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GENETICS

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CORRIGENDA

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Page 7, line 33, read "to be" after "series."
Page 8, line 34, before "highest" read "lowest or."
Page 8, line 37, before "highest" read "lowest or."
Page 14, line 21, for "33" read "3."
Page 19, line 12, for "1914" read "1915."
Page 113, line 2, read "due to" after "undoubtedly."
Page 113, line 32, for "defintie" read "definite."
Page 406, legend table 8, for "F_1 duplex ski ♀ × wild type ♂." read "Backcross, duplex ski ♂ × F_1 wild-type ♂."
Page 569, line 9 from bottom for "n" read "in".

AUGUST WEISMANN

(Frontispiece)

August Weismann, who is entitled to be considered one of the godfathers of genetics, was born at Frankfort-am-Main, January 17, 1834. Like most biologists of his day he was schooled in medicine; and, after taking his degree at Göttingen in 1856, he spent two years acting as personal physician to Archduke Stephen of Austria. Fortunately, the practice of medicine gave him no intellectual satisfaction, and in 1863 he began to prepare for research in embryology and morphology under Leuckart. Three years later he accepted a professional appointment in zoology at Freiburg-im-Breisgau, where he remained until his death on November 6, 1914.

As is well known, Weismann was afflicted with an eye trouble, which prevented him from working continuously at the microscope. Nevertheless there were periods when he was able to give attention to laboratory investigations, and his output of discoveries at these times shames many a biologist who had good eyes but saw not. In particular, one may mention his embryological work on certain species of diptera and of crustacea, his study of the origin of the germ-cells in Hydromedusae and his observations on variation and heredity, with insects and small mammals as material.

This enforced inactivity of hand and eye proved to be something of a blessing. Frau Weismann (geb. Maria Gruber) became her husband's reader, amanuensis, and collaborator; and, for the twenty years of her married life, served as recording angel for the thoughts of his active brain. The result was a continuous flow of biological generalizations that spread the fame of the Freiburg professor throughout the world. A just fame it was; for here was no studio philosophy, but the philosophy of a trained experimentalist, a keen observer, who, by force of circumstances, was compelled to ponder the facts of the organic world slowly and carefully.

Haeckel and Weismann became the Peter and Paul of Darwin; and, as is often the case with apostles, they rather overrode the ideas of the master. From Jena came the thunders of the monist; from Freiburg came no less positive utterances on the all-powerfulness of natural selection,—a neo-Darwinism of somewhat different character from that of Wallace in England.

But Weismann was not content to be a herald. He was a constructive genius. He began to show the independence of his ideas in his work on geographical isolation, which was a broader consideration of the whole

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subject than had appeared since the Origin of Species. In it one finds the presentation of an idea which was to become an important fixture in biological theory,—the idea of crossing as a means of producing material from which came the survival of specific types. In later papers, Weismann elaborated the conception, postulating the immortality of the protozoa and accounting logically for the evolution of the metazoa, with progress as the result of the development of sexual means of multiplication and death the payment by the individual. These daring proposals caused a clash with MAUPAS, who believed that conjugation was essential to protozoan life; but WEISMANN maintained his position firmly, and lived to see the argument of MAUPAS broken down by the experiments of WOODRUFF.

Though biology owes much to WEISMANN'S keen, analytical mind, which illuminated every question it considered, the deepest impression on the science was made by his masterly essays on The Germplasm as a Basis for a Theory of Heredity. Briefly, the theory which he defended in this work states that inheritance is from germplasm to germplasm is unbroken continuity from the beginning of life. Heredity is simply the consequence of the fact that offspring are parents are composed of some of the same materials, the bodies of each being outgrowths from a part of the germplasm set apart for such purpose. Variation and selection occur within the germplasm, and amphimixis furnishes a mechanism whereby various permutations and combinations of the germplasm can be brought together. Doubtless some portions of this theory will not stand the test of time, such being the usual fate of theories; yet, in its essential features, there is such a close approximation to real truth that it will remain of value should it be shown ultimately that characters acquired by the soma occasionally are inherited. And certainly there is no reason, at the present time, to question the validity of many strictly Weismannian concepts,—the continuity of the germplasm, changes in the constitution of the germplasm as the primary cause of variation, the chromosomes as the essential part of the heredity mechanism, the reduction of chromatin content in the maturation of eggs and sperm, and the linear arrangement of the "determinants" of body characters in the chromosomes.

One can maintain, with little fear of contradiction, that no biologist, other than DARWIN, erected such an elaborate superstructure of speculation upon the meagre facts of his time, made so many predictions from the logical requirements of the situation, and had such a goodly portion of these predictions verified by later investigations. But, if a suggestion is not out of place, it is not for these things, solely, that the student should turn more often to the work of WEISMANN. It is for the keen analysis of scientific data by which he demolished the current case arguments of Lamarckism. No greater power of analytic resolution, no greater clarity of exposition is found in the annals of biology.

Though he wrote numerous technical papers, WEISMANN is chiefly known for his general treatises, of which the following are the most important: Über die Berechtigung der Darwinschen Theorie (1868); Einfluss der Isolirung auf die Artbildung (1872); Studien zur Descendenz-Theorie, 2 Bde. (1875–6); Die Dauer des Lebens (1882); Die Kontinuität des Keimplasmas als Grundlage einer Theorie der Vererbung (1885); Das Keimplasma (1892); Die Allmacht der Naturzüchtung (1893); Neue Gedanken zur Vererbungsfrage (1895); Germinal-Selektion (1895); Vorträge über Deszendenz-Theorie (1902); Die Selektions-Theorie (1909). The outstanding works available in English are: Essays upon Heredity and Kindred Biological Problems (vol. I, 1889; vol. II, 1892), translated by POULTON and SHIPLEY; The Germplasm (1893), translated by N. PARKER; and The Evolution Theory (1904), translated by J. A. and MARGARET THOMSON.

The portrait reproduced here is from a photograph taken in the Atelier Elizabeth, Munich, a copy of which—together with the autographic signature—was kindly lent for this purpose by a former student and assistant of WEISMANN, Professor ALEXANDER PETRUNKEVITCH of YALE UNIVERSITY.

The reproduction of this portrait has been made possible through a gift received from MRS. CHARLES CARY RUMSEY, who, like her mother, MRS. E. H. HARRIMAN, founder of the EUGENICS RECORD OFFICE, has been closely identified, through constant active encouragement and support, with the progress of human genetical research in this country during more than a decade past.

THE EFFECT OF SELECTION FOR EYE FACET NUMBER IN THE WHITE BAR-EYE RACE OF *DROSOPHILA MELANOGASTER*[1]

CHARLES ZELENY

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Received June 13, 1921

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[1] Contribution from the Zoölogical Laboratory of the UNIVERSITY OF ILLINOIS, No. 196.

I am indebted to DR. JOSEPH KRAFKA, JR., for the greater part of the routine work of the present experiments, and to MR. D. H. THOMPSON for help in the arrangement of the data for publication.

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INTRODUCTION

The present report is one phase of an intensive study of the germinal and environmental factors affecting eye-facet number in Drosophila.[2] It gives the results of forty-two generations of selection for high facet number on the one hand and low facet number on the other. The objects in view are the determination of (1) the character of the germinal diversity present in the stock at the beginning of selection, and (2) the manner of appearance of germinal changes during the course of selection. The results obtained are to be considered not only for their independent value, but also because they form a necessary link in the solution of the general problem of the explanation of the character differences. The general viewpoint of the writer has been that an analysis of a single character with a view to the correlation of the different processes involved in its production is of the greatest importance at the present time. There is no attempt to deny that there has been a need for the emphasis on a clear distinction between the problem of the mechanism of transmission of "genes" and the problem of the ontogenetic development of characters. On the other hand, it seems to the writer that at present there has developed a greater need for the attempt to understand each of these processes in relation to the other. There can be no full understanding of the mechanism of transmission unless there is an understanding of the sort of thing that is being transmitted, and the reverse is equally true.

In some earlier work started in 1914 (ZELENY and MATTOON 1915) it was shown that bar eye, which at that time was a recent mutation from full eye, possessed germinal diversity as regards the factors affecting facet number. Three successive selections for high facet number increased the mean number from 98.0 to 139.5 and three similar selections for low facet number decreased it to 83.7. Significant progress was noted in each of the three selections in both high and low lines. While the selections were not carried on long enough to make possible a clear demonstration, the fact that regression toward the mean of the parental generation increased with successive selections made it seem probable that a limit to the progress of selection would eventually be reached. The same fact made it seem probable that the effectiveness of the selection was due to the sorting out of original differences in germinal composition.

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[2] The reports already published are given in the list of cited literature at the end of the paper.

H. G. MAY (1917) continued the study in this laboratory during the academic year 1915–1916. His first series, with vestigial-winged bar, died out at the end of three generations on account of high sterility. Selection was effective for one generation but failed to produce further effects in the second and third generations. Reverse selections in low lines were not effective. In a second series long-winged bar flies were used. Selection was effective for the six generations during which it was carried on, though in the low line most of the high flies were eliminated by the first three selections. Crosses between the high and low lines indicated that the hereditary factors involved in the difference between the two were not sex-linked. Outside of the selection results an important demonstration of reverse mutation of bar to full was made.

MAY'S data on the effects of selection were not as conclusive as one might wish because of sterility and the high variability in facet number due to uncontrolled environmental factors. His results indicated the probability that temperature is an important factor in this connection. Accordingly the working out of this problem was suggested to E. W. SEYSTER in 1916. His work was incomplete at the time he left for war service, but the results were published in 1919. In the meantime, JOSEPH KRAFKA, JR., took up the problem, and his more complete analysis of the temperature effect was published in 1920. It is clearly demonstrated by these experiments that temperature has a pronounced and definite effect upon facet number. Stated very generally, there is approximately an increase of 10 percent in facet number for each *decrease* of one degree centigrade in temperature except near the temperature limits, where complicating factors enter.

At the same time that these temperature experiments were begun, it was decided to carry on the other phases of the study under as accurate temperature control as possible. Under such conditions a series of selection experiments on a red bar-eye stock, in which all individuals were derived from a single mating containing a single bar gene was started in 1916, and in 1917 a series on white bar eye. The first of these will be reported in a separate paper. The latter is the subject of the present report. White bar was used because it was desired to duplicate an experiment which had previously been carried on by one of the students in the laboratory with results strikingly different from those in our other selection series. The data he obtained, however, have since that time not stood the test of critical analysis.

The experiments recorded in the present paper demonstrate clearly that selection, by taking advantage of the large mutations, rapidly effects an upward change to full eye and a downward change to ultra-bar. The presence of selection and its direction, however, have no effect on the character or rate of the mutations. Selection merely preserves them if they happen to be in the proper direction. Since these mutations to full eye and to ultra-bar are discussed elsewhere (ZELENY 1920 a, 1921 a) the present report will be confined to a discussion of other germinal differences unless the former are specifically mentioned.

If full eye and ultra-bar are set aside as they appear and attention is confined to the remaining individuals, the results demonstrate the existence of distinct germinal differences in the original unselected population. The low-facet factors of pronounced effect are rapidly eliminated in the high line and the high-facet factors in the low line. Factors of smaller degree are protected for a time from the action of selection by non-heritable fluctuations, but eventually they also are eliminated. Such eliminations take place in the low line following the 12th generation and in the high line following the 25th generation. A sex-linked lethal factor affecting facet number in single dose appears in the 21st generation of the high line and persists for several generations. It causes a rise in the mean facet value of the females in these generations.

Selection thus merely exercises a sorting effect and further progress after the preliminary sorting is completed is confined to new mutations whose origin is independent of selection.

MATERIAL

Full eye

Normal wild *Drosophila melanogaster* has a large oval, so-called "full eye" (figure 1 F). The average number of ommatidia as indicated by ten facet counts of each sex in stock number 345 is 810.6 in the females and 849.8 in the males at a temperature of 27° C. The data are given in table 1 under "full eye."

TABLE 1

Eye-facet number in full-eye stock number 345 and white bar unselected stock number 133-7.

	FULL EYE		WHITE BAR EYE	
	Females	Males	Females	Males
Number of counts	10	10	488	441
Mean of facets	810.6	849.8	58.8	111.4
Lowest facet number	632	700	21	35
Highest facet number	924	980	134	314
Mean in factorial units[3]	+26.67 ± 0.24	+21.15 ± 0.17	0.00 ± 0.10	0.00 ± 0.13
Standard deviation in factorial units	1.11 ± 0.17	1.12 ± 0.17	3.12 ± 0.07	3.91 ± 0.09
Lowest individual in factorial units	+24.22	+19.12	−9.68	−10.72
Highest individual in factorial units	+28.02	+22.49	+8.75	+11.11

Origin of bar eye

In an experiment involving rudimentary- and long-winged flies and not concerned with eye characteristics, TICE (1914) found a single male with a reduced number of ommatidia. The eye facets were restricted to a vertical band or bar of irregular outline (figure 1 B). This male was crossed to a normal full-eyed fly, and the daughters were back-crossed to their father. From the offspring of this back-cross there were derived individuals like the original mutant male and they constitute the so-called "bar-eye" race. Bar eye is a sex-linked character, and it is evident from its origin in a single male that all the future bar-eye genes are derived from the single original one. The gene is located at 57.0 on the chromosomal scale developed by MORGAN and his co-workers. It is spoken of as a dominant mutant in the original paper, but the heterozygotes are intermediate in facet number between bar and full eye (figure 1 Z). Using the factorial scale as applied in the present paper, the heterozygote is nearer to full eye than to bar. The facets of bar eye are divided into an upper and a lower group by a constriction slightly below the middle of the eye. It is more pronounced in low than in high bar, and in some cases the two parts of the eye are completely separated.

Bar-eye stocks

A few months after the establishment of the stock, Professor MORGAN kindly sent me some individuals which served as the basis of my stock.

---

[3] For the meaning of factorial units see page 13 and following.

In September 1914, less than a year after the appearance of the original mutant, this stock was used as the starting point of the selection series described by ZELENY and MATTOON (1915). The facet counts in the unselected stock at this time averaged 65.06 in the females and 98.04 in the males. In factorial units, according to the system and scale used in the present paper, these values are +1.50 for the females and −0.50 for the males. The sexual dimorphism indicated in the facet counts was noted throughout the various series of experiments, but it has been shown to vary so much in degree that the method of reduction of the female and male values to a common basis by the use of a constant factor, which was employed in the first paper, has not been followed in the later tabulations. The values for the two sexes in factorial units as given above show that the sexual dimorphism at the given time was different from that in the unselected white bar stock which is used as the standard of reference. The females are relatively higher, and the males relatively lower. As described[4] in a paper now in press, there is

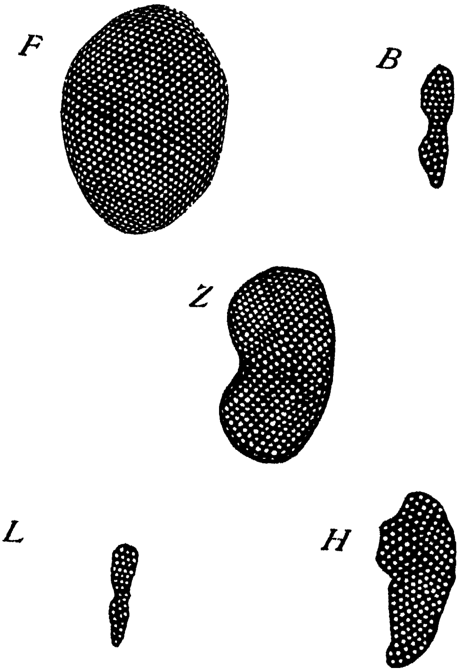

FIGURE 1.—Full and bar eyes. Females. F = full eye. B = bar eye of white unselected stock number 127. Z = heterozygote of full × bar. L = selected low-bar eye. H = selected high-bar eye.

also a pronounced decrease in the dimorphism during the course of selection with inbreeding (ZELENY 1921 b).

The vestigial bar used by MAY (1917) in one of his selection series was obtained from the same stock by crossing with a vestigial-winged full-eyed race. In MAY'S other series the bar stock was used directly.

In 1916 my stock was divided into parts, each of which received a distinct designation. They were carried on separately with a view to determining any divergence of the mean values that might occur under the conditions. One of these stocks, number 31, was later used as the starting point of red-bar selection lines A and B, which are to be described in a separate paper. Another one, number 23, was used in producing the white bar stock which served as the basis of the experiments about to be described.

It was desired in the present selection series to duplicate as nearly as possible the material used in a previous set of experiments. Red bar, number 23, and white full-eye stocks were carried in the laboratory without selection or contamination. During the summer of 1917 they were kept in a constant-temperature room at about 25.6° C. Males of red bar, number 23, were mated to females of white full-eye on August 6. The F_1 offspring were the expected red heterozygous females and white full males. These gave in F_2 the expected non-crossover and crossover classes. The white heterozygous bar crossover females and white bar crossover males were mated in mass, and their offspring (F_3) were treated as follows. The two lowest pairs, a 19-facet female and 31-facet male, and a 21-facet female and 39-facet male, and the two highest pairs, a 141-facet female and a 202-facet male, and a 177-facet female and a 211-facet male, were eliminated and used for a separate test. Of the remaining individuals a random sample was preserved for a count and the rest were mated in mass, giving the pure white bar stock (F_4) which served as the starting point of the selection series.

It is to be noted that several red bar males were used in the original cross with white full, so that several bar genes were present at the start. This condition is to be contrasted with that in another series, described in a separate paper, in which a single bar gene was introduced.

METHOD

Food

The banana was put into an Arnold sterilizer for 45 minutes, sealed in air-tight Mason jars and inoculated with yeast as needed. Uniformity in food conditions was by no means obtained, and some of the variability in facet number is undoubtedly due to this source. A technique making possible a better food control is highly desirable in future work of this character.

Contamination

A special effort was made to avoid contamination, because the study involved the origin of mutations as well as the effect of selection. The evidence that no contamination occurred is similar to that given by MAY (1917), and need not be repeated here except to state that the precautions observed were even more rigid than his, and that no case of contamination was discovered.

Temperature control

In view of the marked dependence of facet number upon temperature as demonstrated by SEYSTER (1919) and KRAFKA (1920), great care was taken to keep the material at a constant temperature throughout the experiment. The departure from 27.0° C did not exceed 0.5° during the whole course of the selection. The details of the temperature control as given by ZELENY (1920 a, pp. 294–296) apply to the present data.

Selection procedure

Brother-and-sister matings were made as consistently as possible. In a few cases death or lack of fertility compelled a resort to cousin matings to insure the continuity of the experiment. Figures 2 and 3 give the pedigrees for the low- and high-selection lines respectively. The selection generations are given in the first column and the catalog numbers of individual matings in the succeeding columns. The original selections naturally came from the mass stock culture. In the low direct line the only exceptions to brother-and-sister matings were in the first generation, which had a cousin mating, and in the fifth, which was a mass culture. In the high direct line the only exception is in the 21st generation. Among the matings not in the direct line, one in the 20th generation of the low selection, and one each in the 27th, 28th and 36th generations of the high line are not brother-and-sister matings.

As a matter of general routine, it was planned to mate the lowest (or highest) male in each generation with the lowest (or highest)female. The next-lowest (or highest) were then mated, and so on. If the highest pair gave a considerable number of offspring, these were used for the next selection. If they were sterile, or gave only a few offspring, the next-highest pair was chosen, and so on. While "performance" did not enter into the plan of the procedure as originally outlined, it did enter as an element in selection in a few places because in case the offspring of the lowest (or highest) pair were only few in number, they were more frequently chosen for the continuation of the line when their values were in the direction of the selection than when they were strikingly in the other direction.

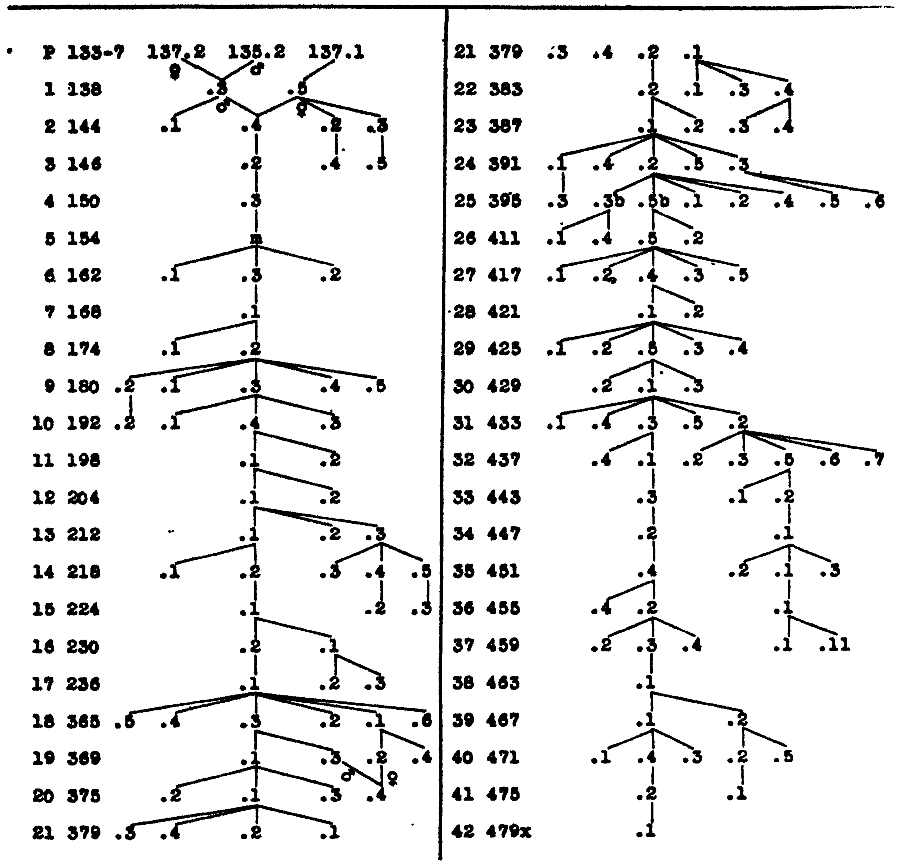

FIGURE 2.—Pedigrees in low line. The generations and culture numbers are given. The direct line is the continuous vertical one. All matings are of brothers with sisters except where male and female parents are indicated as coming from two sources.

At times the fertility was low, and it was necessary to mate as many as twelve or even more pairs to insure continuity of the lines. The offspring of the sib-matings were recorded, and the data are included in the tables labeled "all matings." In every case, however, the "direct-line" matings are separately tabulated.

Recording of the data

The data were recorded in notebooks paged consecutively, and entries were made in day book fashion. All the records are referred to by page number with a decimal corresponding to position on the page. For instance, number 144.2 refers to the data recorded on page 144 in the second column from the left. The bottles of flies had the same numbers. Each bottle and each corresponding entry in the notebook had attached to it the source of the parents, their facet numbers, date of mating, food-culture number, date of removal of parents, temperature, and bottle sequence in case more than one bottle was used for the same pair. After a considerable experience with both cards and bound notebooks, the writer favors the latter if a tendency to crowding can be avoided. It is his experience that the day-book scheme for the original data causes the recording of more details than do other systems because there is no need of hunting for the proper place of entry.

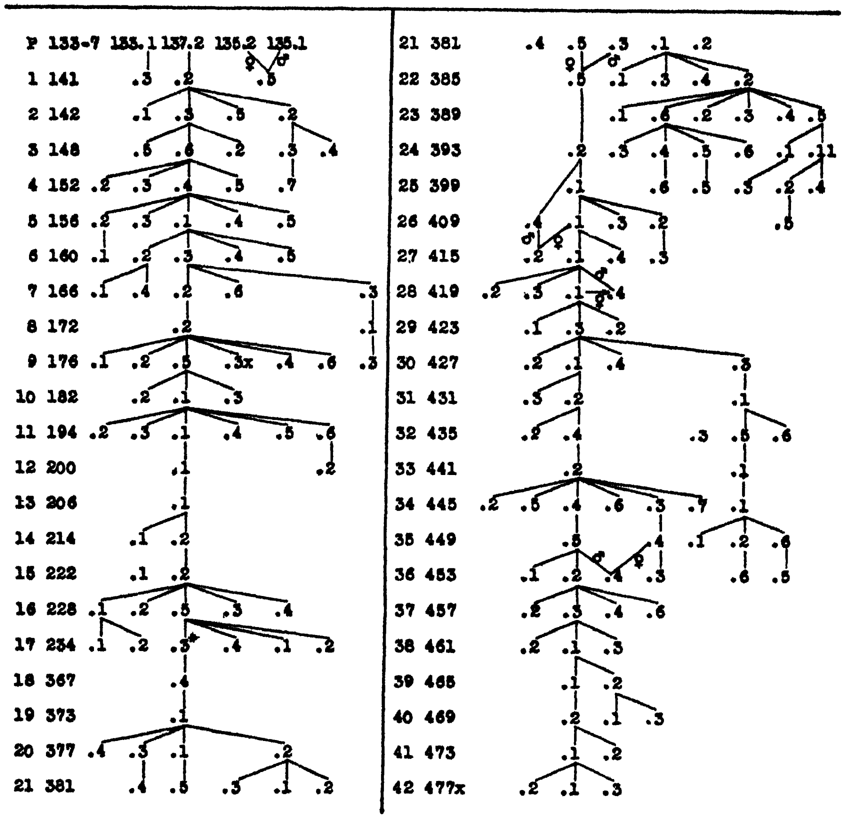

FIGURE 3.—Pedigrees in high line. Arrangement as in figure 2.

The degree of selection

In the low direct line the average difference between the means of the selected parents and the means of the populations of which they were a part was −1.71 factorial units. Including the sib-matings, the average was −1.79.[3] Due to the exigencies of the experiment, largely a matter of low viability, five of the matings in the direct line and one generation when all matings are included, were actually high selections, the means of the selected pairs being higher than the mean of the population. In one generation, also in both direct and all matings, there was a resort to a mass selection. In the remaining 36 generations of the direct line and 40 generations of all matings (those having a minus selection) the average degree of selection was −2.02 factorial units for the direct line, and −1.87 for all matings. The values for the individual generations are given in tables 26 and 27 and figures 122 and 123.

In the high direct line, the average difference between averages of the grades of the selected parents and the means of the populations of which they were a part was +2.16 factorial units. Including the sibs, the average was +2.10. In three of the generations of the direct line and in two of "all matings," there was a minus selection. The average degree of the remaining 38 or plus generations of the direct line, was +2.41 factorial units, and of the 39 plus generations of all matings, +2.30. The values for the individual generations are given in tables 28 and 29 and figures 124 and 125.

Synchrony between low and high lines

It was deemed desirable to keep the low and high lines of any generation as nearly synchronous as possible. This was done with the object of aiding in the elimination of differences due to food or other factors not otherwise controllable. The degree of success of the attempt at synchrony is shown in table 2 which gives the dates of the matings for all the generations in both low and high lines. It will be noted that the 23rd generation of the high line is skipped in order to bring the two lines to equal dates of mating. A reference to the pedigree chart (figure 3) shows the necessity for this procedure. It was due to the loss of the prospective direct line and the substitution of a prospective sib-mating for the lost one. In the discussion of the experiments, as well as in the tables, it seems best to call the high-selection generations beginning with the 23rd by generation numbers one higher than the actual so as to make them agree in time with the coördinate low-selection generations.

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[3] For the meaning of factorial units see p. 13 and following.

TABLE 2

Synchrony between low and high generations. White bar selection, 27° C.

	LOW	HIGH		LOW	HIGH
P	1917 IX 26	1917 IX 26	22	1918 VIII 13	1918 IX 11
1	X 9	X 13	23	VIII 26	
2	X 23	X 25	24	IX 25	IX 24
3	XI 3	XI 6	25	X 11	X 11
4	XII 1	XI 17	26	X 22	X 22
5	XII 14	XII 17	27	XI 2	XI 2
6	1918 I 1	XII 28	28	XI 14	XI 21
7	I 15	1918 I 14	29	XII 5	XII 5
8	II 12	I 26	30	XII 20	XII 20
9	III 1	II 8	31	1919 I 1	1919 I 8
10	III 13	II 22	32	I 23	I 25
11	III 22	III 7	33	II 6	II 11
12	IV 5	III 23	34	II 25	II 27
13	IV 16	IV 10	35	III 11	III 11
14	IV 26	IV 25	36	III 25	III 27
15	V 11	V 11	37	IV 8	IV 8
16	V 24	VI 4	38	IV 22	IV 24
17	VI 4	VI 15	39	V 14	V 7
18	VI 19	VII 4	40	V 26	V 21
19	VII 4	VII 16	41	VI 5	VI 12
20	VII 16	VII 26 VIII 2	42	VI 20	VI 25
21	VIII 1	VIII 26			

Times of first matings in the direct line in each generation, except the last in which the dates of the facet counts are given.
Average length of generation in days, low line = 12.7, high line = 12.8.
Total period in days, low line = 533, high line = 538.

Accuracy of facet counts

The accuracy of the facet counts was tested at various times to see if it was sufficient for the purposes of the experiment. Most of the facets are arranged in rows, and it is easy for the eye to follow them. Some are irregular in arrangement, and occasionally one or two facets are of small size. The counts cannot, therefore, be absolutely correct under the conditions of the experiment which involved the handling of such large numbers of individuals. They are, however, of sufficient accuracy for the purpose in view, as shown in table 3, which includes an ordinary routine count and a recount made several weeks later by JOSEPH KRAFKA, JR., who is responsible for nearly all of the counts made in connection with the present experiment.

TABLE 3

Accuracy of counts. Test by JOSEPH KRAFKA, *Jr.*

	FEMALES		MALES	
	Original	Recount	Original	Recount
	1918 I 21	1918 III 3	1918 I 21	1918 III 3
	34	35	32	33
	37	36	39	37
	38	38	40	40
	41	40	40	41
	41	41	40	41
	42	42	41	43
	42	42	41	43
	43	42	47	45
	43	43	49	48
	45	45	52	51
Average	40.6	40.4	42.1	42.2

THE TABULATION OF FACTORIAL VALUES FOR FACET NUMBERS[4]

General statement

In working up the data, it became evident that the demands of the biological analysis were not adequately met by the system of arrangement in classes with equal facet numbers. It seemed desirable in so far as is possible, to express relations directly in terms of factorial units affecting facet number rather than in facet numbers. Direct experiment with one factor, temperature, had demonstrated that its action is not one of accretion, as this system demands, but one of proportionate action. The result depends upon the amount of material acted upon as well as upon the temperature. Assuming that the action of other factors follows the same principle, the geometric or logarithmic mean and the dispersion of the logarithms of the facet numbers should form the basis of the biological analysis. That this is a proper procedure is further indicated by the marked positive skewness of the variation curves of facet number as plotted on the arithmetic basis and the normal form of the logarithmic curves.

The particular form which the analysis takes in the present paper was determined primarily by the need of a procedure which would enable one to visualize the biological significance at all points. The following discussion has that object in view.

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[4] A brief statement of this method of tabulation is given in a previous paper (ZELENY 1920 b).

For reasons to be stated presently, in dealing with a stock averaging 30 facets as compared with one averaging 300 facets, it seemed that the unit value should not be one facet for both but rather that a change of one facet at the mean in a 30-facet stock should be recognized as representing the same factorial value as a change of ten facets at the mean in the 300-facet stock, and a corresponding principle should apply within the range of a single stock. In the ordinary determination of variation constants this principle is, of course, recognized in the formula for the the coefficient of variation in which $\text{C.V.} = \frac{\sigma}{\text{M}} \times 100$, or the standard deviation per unit of the mean multiplied by 100. The formula, however, makes the reduction only for the population as a whole. It does not make any provision for comparisons within the range of a single population and therefore does not meet the demands of graphic representation.

In the tabulation with equal facet values for the different classes, the difficulties connected with the comparison of stocks with greatly different means can be met for purposes of tabulation of the data by use of the coefficient of variation as given above. In graphic representation, the different populations can be brought to a comparable basis by using class ranges differing for the different stocks and equal for any stock to a certain definite percent of the mean of that stock. Thus for a stock with a mean of 30 facets, classes each of which has a range of 33 facets or 10 percent of the mean of that stock, might be used. Within the population of each race, however, there would still be a discrepancy between the individuals with high and those with low values, because the class range in each stock is too low for the individuals with high facet number and too high for those with low facet number.

As given later, biological considerations in connection with eye-facet formation, and the same undoubtedly hold for many other similar organs, lead to the belief that environmental and presumably germinal factors do not act by accretion, but that the effect is upon the rudiment of the organ as a whole. A unit factorial change in a 300-facet race yields ten times as much facet change as in a 30-facet race, because it has ten times as much material to act upon.

Considering this hypothesis of the proportionate relation of factors to somatic characters to be correct, a population which has a normal variability curve as regards factors will have a positive skewness as regards the eye-facet characters. The obvious correction for this condition is to make the class ranges vary in accordance with the hypothesis. If each class range is a definite fixed percent of the mean of *its* class, then throughout the whole range of variation the class ranges are comparable, and each class has the same factorial value.

The variation data are thus put into factorial units and not eye-facet units, and the various constants can be determined in the ordinary way. Suppose that the class facet ranges are in every case equal to ten percent of the mean class facet values, which is the amount produced by a change of one degree centigrade, and a factorial unit is one that produces a ten percent change in the facet value of a class; then, according to the hypothesis, all the factorial units have the same factorial value though their facet values vary with the means of the classes. Starting with some arbitrary point, for instance the mean facet value of the unselected stock in a selection experiment, and calling this point zero, then every facet value has a definite factorial value equal to plus or minus a certain definite number of factorial units from the zero point. Five units above the mean is equal to five units below the mean, and similarly everywhere in the scale, while a departure of +5 or +50 facets under the ordinary system of tabulation does not have the same factorial value as −5 or −50 facets. The standard deviation, as determined by this method, is expressed in factorial units and serves directly as a coefficient of variation strictly comparable in all cases, regardless of the mean values of the different stocks that may be compared.

Validity of the method

The validity of the method is, of course, dependent upon the biological assumption that eye-facet value is such a function of factorial value as is indicated. The evidence in favor of this view will now be considered.

Proportionate action *versus* accretion

It is a common principle of embryology that a changed condition does not act by accretion, i.e., by the addition or subtraction of individual parts without affecting the rest. On the contrary, the action is upon all the pre-existing parts of the organ. The result then depends not only upon the strength of the new agent, but also upon the reaction capacity of these pre-existing parts. Since a new factor, f, acts upon the whole complex, the value of the pre-existing mechanism, m, is a factor in the result as well as f. This general statement applies to both germinal and environmental factors. For instance, it is clear that any particular factor affecting facet number is not something which can of itself produce facets, nor is it a certain amount of substance with a capacity limited to the production of a fixed number of facets.

Germinal factors

As regards germinal factors, it is clear that the chromosomal mechanism distributes them equally to all the cells of the body. Limiting the consideration to a single effect of a particular factor, if at the stage at which this factor can function there are eight cells in the reacting region in one individual and only four in another, the resultant effect is twice as great in the first as in the second. This is a crude statement of the case because the action of the different factors is no doubt to a large extent simultaneous, but it makes clear the principle involved.

Environmental factors

That the theory of proportionate action is a closer approximation for the temperature effect than the theory of action by accretion is demonstrated by the experiments of SEYSTER and of KRAFKA. SEYSTER (1919) has shown that in the bar eye, facet number decreases with increase in the temperature at which the larvae of Drosophila are reared. This decrease follows VAN'T HOFF'S law if an inhibitor of facet number is assumed as the effective agent upon which the temperature acts. KRAFKA (1920) has demonstrated that this law applies to ultra-bar as well as to bar eye, and that for the different stocks the effect of a degree of change in temperature is roughly proportional to the mean facet value of the stock, and the same is approximately true for the effect throughout the range of a single stock. Table 4 gives the results of one of his experiments with ultra-bar, low-selected bar and unselected bar. The facet numbers for 15° and 25° are given, and the facet differences for the three stocks are respectively 26.3, 114.8 and 149.3. Representing the effect of a ten-degree difference for ultra-bar as unity, low-selected bar has 4.4 times and unselected bar 5.7 times this difference. It is obvious that difference

TABLE 4

Facet values and factorial values at 15° and 25° in ultra-bar, low bar and unselected bar.

STOCKS	FACET VALUES				FACTORIAL VALUES			
	15°	25°	Differences	Ratios $=\frac{X}{B'U}$	15°	25°	Differences	Ratios $=\frac{X}{B'U}$
Ultra-bar $=B'U$	51.5	25.2	26.3	1.0	− 0.83	−7.86	7.03	1,0
Low bar	189.0	74.2	114.8	4.4	+12.17	+2.79	9.38	1.3
Unselected bar	269.8	120.5	149.3	5.7	+15.72	+7.73	7.99	1.1

in facet value is not a good measure of the value of the temperature factor.

On the other hand, if the facet values are reduced to factorial values, the differences between the 15° and 25° values are 7.03 factorial units for ultra-bar, 9.38 for low bar and 7.99 for unselected bar. The last two values are respectively 1.3 and 1.1 times the first, while the corresponding facet values are respectively 4.4 and 5.7 times the facet difference for ultra-bar. The factorial units, therefore, give a much closer approximation than the facet numbers.

A change of one facet is, therefore, not of equal factorial value at different points in the variation scale as far as temperature is concerned. A plotting of the data using facets as the units does not give a uniform factorial scale. Suppose temperature to be the only factor causing variation in the facet number of a particular stock, and knowledge of the actual temperatures involved in the production of a particular population to be lacking, and it is desired to derive the value of the temperatures from the facet values. Obviously the closer approximation would be obtained by the tabulation in which each class has a range equal to a definite percent of the mean of the class. KRAFKA's data show that even in this case the values are not exact, but certainly the error is of a much lower order than that involved in using facets as the units.

Skewness

When eye-facet numbers are plotted on the basis of classes with equal facet ranges, they usually show a very distinct positive skewness, i.e., the mean is higher than the mode (figure 4). Such a skewness may be due to any one or more of a number of causes, but it is not the intention to discuss them here in detail. One evident explanation is that the facet values on that system of plotting do not correspond with factorial values. If we employ the method of factorial units, this skewness disappears in large part and the curve shown in figure 5 is obtained. If the factorial values fluctuate evenly around the mean, the curve should be without skewness when plotted on the basis of those values, but should have a positive skewness if plotted on the basis of classes with equal facet ranges.

If this explanation applies to eye-facet numbers, it is very probable that it applies to similar cases elsewhere. In this connection it is interesting to note that positive skewness is very common in variation data as tabulated by the ordinary method. Some, at least, of these cases may be explained on the same basis as the one under discussion. Separate proof, however, is required in each case.

Zero point

The location of the point of reference or zero point in the factorial determinations at the mean of the unselected population has the additional value of being itself in the zone of normal factorial action. The same can not be said of zero facet number, for it is obvious that the factorial action involved in producing a change from zero to any value above zero is of a different order from that involved in any other change whatsoever within the range of variation.

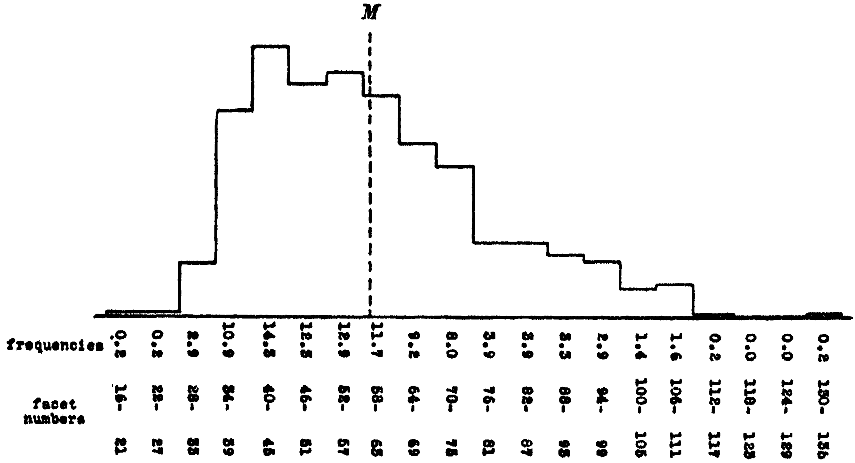


FIGURE 4.—Unselected white bar females arranged in classes each with a range of six facets. 488 individuals. M = mean. There is a striking positive skewness.

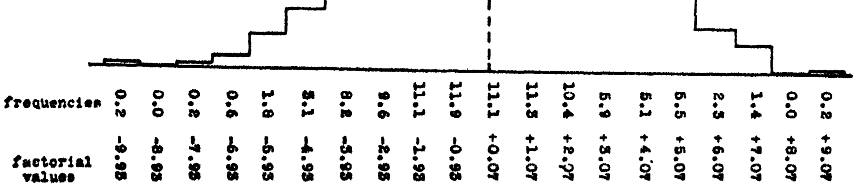


FIGURE 5.—Unselected white bar females arranged in classes each of which has a range of ten percent of the mean facet value of the class. 488 individuals. M = mean. The lower row of figures gives the departures in "ten-percent" factorial units from the mean.

Application to white bar selection

The scale of factorial classes with corresponding facet values as used in the white bar selection lines is given in table 5. The first column gives the mean factorial values of the classes in the females and the third column those in the males. The middle columns give the corresponding facet ranges. The bases of reference or the zero points of the factorial scales are respectively the mean facet value of the females and of the males of the unselected population. For females this value of the zero point is 58.8 ± 0.58 facets and for the males 111.4 ± 1.44 facets. If the facet ratio between the sexes were a constant, it would be possible to reduce the male to the female values or *vice versa*, according to the method followed by ZELENY and MATTOON (1914). The great variation in the ratio in the present material, however, makes it desirable to use separate male and female scales.

Each class has a range in facet value equal approximately to 10 percent of the facet mean of its class. An exact relation of 10 percent would have involved dividing individuals with the same facet value between two classes, but the demands of the analysis are met by the approximation which allows all individuals of a single facet value to be grouped in the same class. An exception was made for individuals with 14 facets which are divided equally between two classes, as part of the procedure in making the departures of the lower classes from the mean as nearly accurate as possible.

Since on the average in the bar-eye races a decrease of one degree centigrade in temperature causes an increase of 10 percent in facet number, the factorial scale may be read as a temperature scale. In other words its units of value are equivalent to degrees centigrade and the difference between any two points may be expressed as so many degrees of temperature. Thus the difference in facet number between ultra-bar and bar eye may be expressed as equal to approximately twelve degrees of temperature or in other words ultra-bar may be changed to the bar level by decreasing the temperature by that amount.

It will be noted that each facet value may be represented as plus or minus a definite number of factorial units from the point of reference, the mean facet value of the unselected population. The determination in factorial units of the mean, standard deviation and other constants is readily accomplished directly as in the ordinary methods. For example, the mean of the males of the 25th generation of upward selection is +4.60 ± 0.16 factorial units, and the corresponding mean of the males of the

TABLE 5

Factorial scales in the white bar selection lines for females (first column) and males (third column) using the means of the unselected population as the zero points and arranging the classes so that each will have a range approximating ten percent of its mean facet value. The corresponding facet values of the classes are given in the middle columns.

CLASS FACTORIAL VALUES FEMALES	CLASS FACET VALUES	CLASS FACTORIAL VALUES MALES
−16.93	10	−23.05
−15.93	11	−22.05
−14.93	12	−21.05
−13.93	13 and 14 (part)	−20.05
−12.93	15 and 14 (part)	−19.05
−11.93	16−17	−18.05
−10.93	18−19	−17.05
− 9.93	20−21	−16.05
− 8.93	22−23	−15.05
− 7.93	24−26	−14.05
− 6.93	27−29	−13.05
− 5.93	30−32	−12.05
− 4.93	33−35	−11.05
− 3.93	36−39	−10.05
− 2.93	40−43	− 9.05
− 1.93	44−48	− 8.05
− 0.93	49−53	− 7.05
+ 0.07	54−59	− 6.05
+ 1.07	60−65	− 5.05
+ 2.07	66−72	− 4.05
+ 3.07	73−80	− 3.05
+ 4.07	81−88	− 2.05
+ 5.07	89−97	− 1.05
+ 6.07	98−107	− 0.05
+ 7.07	108−118	+ 0.95
+ 8.07	119−131	+ 1.95
+ 9.07	132−145	+ 2.95
+10.07	146−160	+ 3.95
+11.07	161−177	+ 4.95
+12.07	178−196	+ 5.95
+13.07	197−217	+ 6.95
+14.07	218−240	+ 7.95
+15.07	241−265	+ 8.95
+16.07	266−293	+ 9.95
+17.07	294−324	+10.95
+18.07	325−358	+11.95
+19.07	359−396	+12.95
+20.07	397−438	+13.95
+21.07	439−484	+14.95
+22.07	485−535	+15.95
+23.07	536−591	+16.95
+24.07	592−653	+17.95
+25.07	654−722	+18.95
+26.07	723−798	+19.95
+27.07	799−882	+20.95
+28.07	883−975	+21.95
+29.07	976−1078	+22.95
+30.07	1079−1191	+23.95

25th generation of downward selection is −7.76 ± 0.11. The effectiveness of the selection is represented by the difference between the two, which is 12.36 ± 0.19. The standard deviations are 1.81 ± 0.09 for the high males and 1.77 ± 0.08 for the low males of this generation. They may be used directly as the coefficients of variation since no correction for mean facet values needs to be made. In the case cited, the difference between the two standard deviations is obviously not significant. As compared with the standard deviation of the unselected males, which is 3.91 ± 0.09 factorial units, there has been a significant decrease in variability.

Other examples

The advantages of the factorial basis for purposes of calculation and tabulation may be illustrated by some further examples.

The determination of a coefficient of dominance

As shown in table 6, the mean facet number of low-bar females in the twenty-fourth generation of selection is 35.1, that of full-eyed females 810.6 and that of the heterozygotes between the two, 399.9. Since the facet values between 35.1 and 399.9 do not have the same factorial value as those between 399.9 and 810.6, the relative strengths of bar and full in determining the value of the heterozygotes cannot be obtained directly. However, by putting these facet values in terms of factorial units of equal value throughout the scale, it is possible to get such coefficients by use of the formulae

TABLE 6

Dominance of bar and full.

FEMALES	CATALOG NUMBERS	NUMBERS OF INDIVIDUALS	MEAN FACET VALUES	MEAN IN FACTORIAL UNITS	FACTORIAL UNITS FROM HETEROZYGOTES	DOMINANCE IN PER CENTS
Low-selected bar (F24)	391.2	129	35.1	− 4.45	+23.89	23.2
Full stock	345	10	810.6	+26.67	− 7.23	76.8
Heterozygotes	769	19	399.9	+19.44		

$$\text{C.D. of bar eye} = \frac{F-H}{F-B} \times 100, \text{ and}$$

$$\text{C.D. of full eye} = \frac{B-H}{B-F} \times 100$$

in which B is the factorial value of bar eye, F of full eye and H of the heterozygote. Assuming uniformity of action throughout the whole range, these determinations give a value of 23.2 percent for the dominance of low bar, and 76.8 percent for that of full eye on a scale in which 100 percent represents complete dominance and 0.0 percent complete recessiveness.

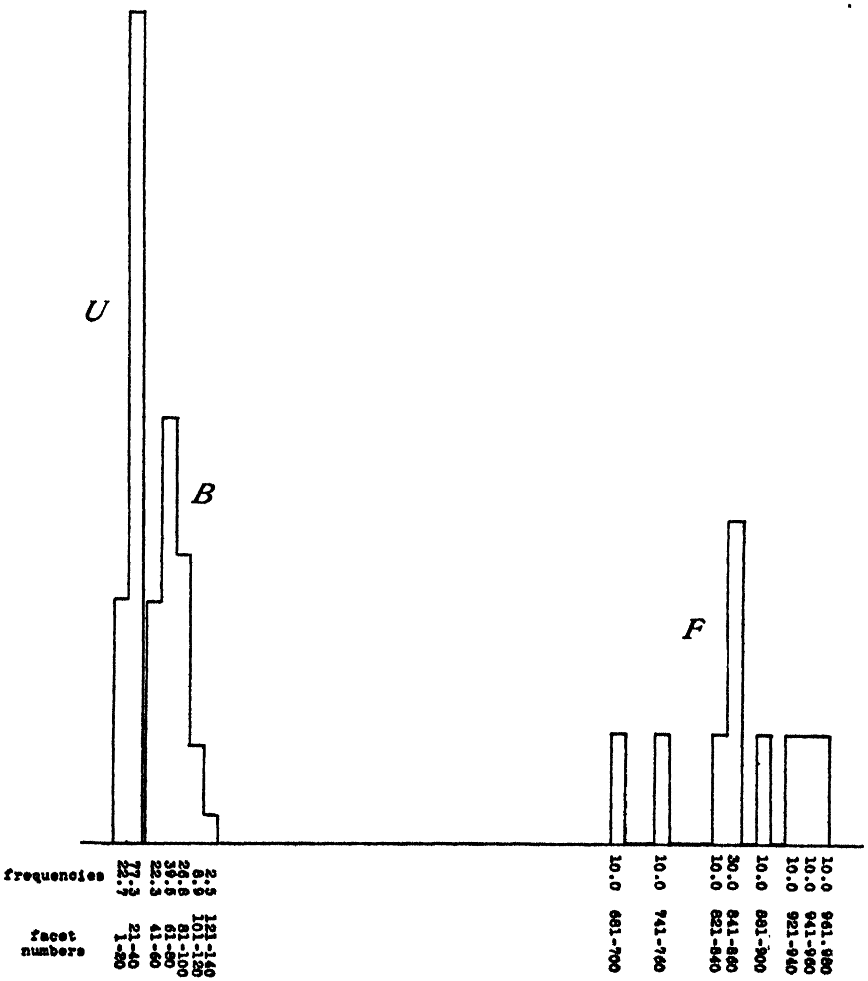


FIGURE 6.—Males of ultra-bar (U), low-bar of the second generation (B) and full eye (F) arranged in classes each of which has twenty facets. All raised at 27° C. Compare with figure 7 which shows the same data arranged in factorial classes.

The comparison of variability in ultra-bar, bar and full eye

Figures 6 and 7 represent variability in ultra-bar (U), bar of the second low-selected generation from which ultra-bar was derived (B), and full

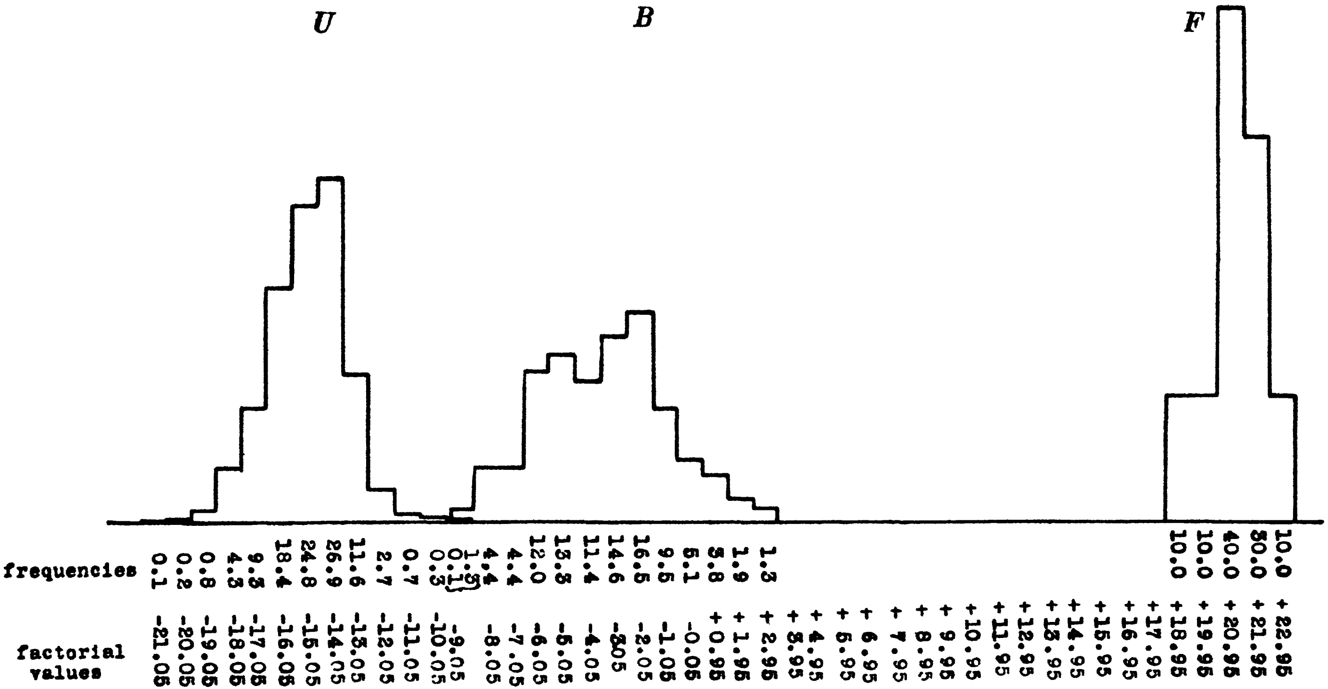


FIGURE 7.—Males of ultra-bar (U), low-bar of the second generation (B), and full eye (F), arranged in classes each of which has a range equal to ten percent of its mean facet value. The lower row of figures gives the departures in "ten-percent" factorial units from the mean of unselected bar.

eye (F). In figure 6 the classes are arranged in the ordinary way so that each has the same facet range. In figure 7, each of the classes has a facet range equal to 10 percent of its mean facet value. The superiority of figure 7 over figure 6 as a true representation of factorial values is apparent.

The comparison of low and high selection lines

Figures 8 and 9 represent the low and high lines of white bar eye after 25 generations of selection. Figure 8 is based on equal facet values for all the classes, and figure 9 has classes each of which has a facet range equal to 10 percent of its mean facet value. Again the superiority of the second representation is apparent.

The representation of mixed populations

Figures 9 and 10 give the variability among daughters of a heterozygous female (from ultra-bar × bar) by a bar male. Heterozygous and bar females are expected. Other crosses show that the two classes overlap, and the same fact is indicated by the distribution in this case. Figure 10 gives the data as arranged in classes of equal facet value. Figure 11 gives them as arranged in classes of equal factorial value. The latter representation is obviously superior for purposes of biological analysis.

DATA

Original data

The importance of the manipulations to which the data are subjected has been emphasized in the last section. The original data before analysis should therefore be available. They have been collected for this purpose in a series of tables, of which one is published here (table 7). The complete tables are on file in the Zoölogical Laboratory of the UNIVERSITY OF ILLINOIS where they are available for study or for photographic reproduction. In each table there are given all the facet counts for the particular generations with which it deals. The uppermost horizontal column gives the generations of selection. The second column contains the catalog numbers of the individual matings as applied to the offspring, the third the catalog numbers of the matings from which the parents were derived, the fourth the facet counts of the female parents and the fifth the facet counts of the male parents. Under each mating the daughters and sons are listed in separate columns. The vertical column at the left gives the facet counts and in their proper places are listed

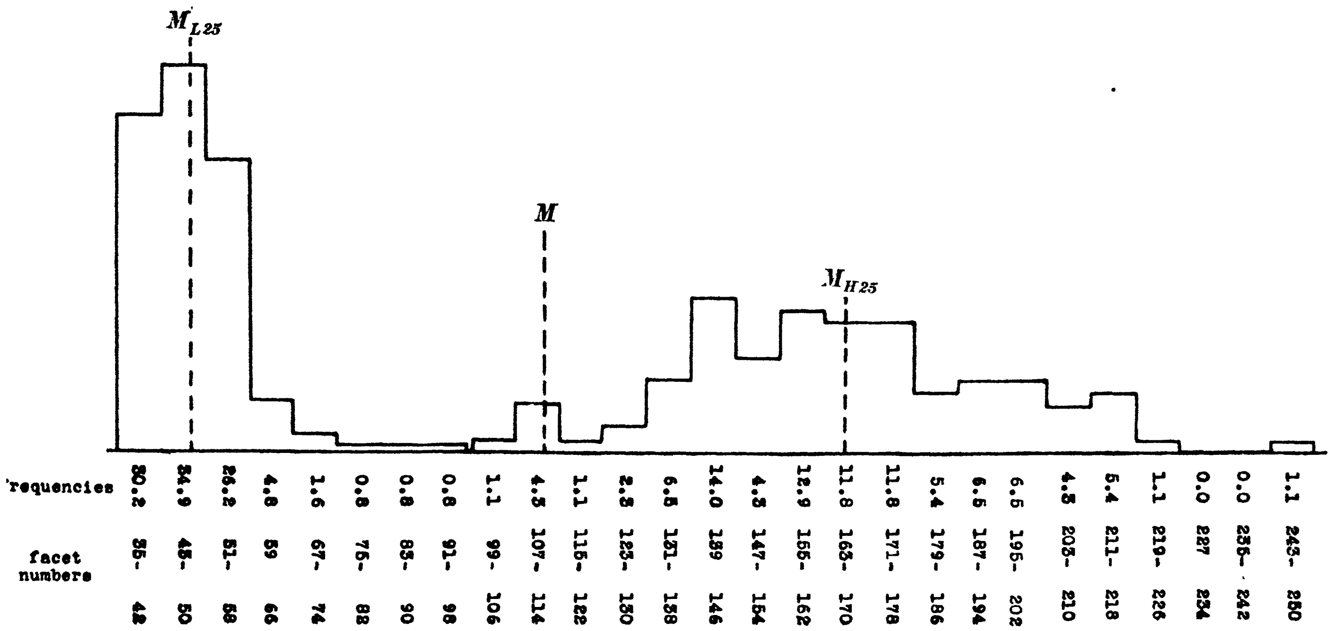


FIGURE 8.—Low-selection and high-selection males of the 25th generation arranged in classes each of which has a range of eight cets. 126 individuals in low-selection line and 93 in high-selection line. M_{L25} = mean of low-selection line = 48.2 facets. M_{H25} = mean high-selection line = 166.1 facets. M = mean of the unselected males = 111.4.

TABLE 7

Tabulation of original data. Eye-facet numbers. White bar selection. Low line. Temperature =27° C. Banana yeast food. September 12, 1917 to July 15, 1919. (Sample table.)

GENERATION	F 37												F 38		F 39					
Mating	459.3		459.1		459.11		459.2		459.4		Total		463.1		467.1		467.2		Total	
Parents from	455 2		455 1		455 1		455 2		455.2				459 3		463 1		463.1			
Facets ♀	34, 34		33		35		34		33				33		35		38			
Facets ♂	42		34		35		39		41				40		36		43, 43			
Offspring	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
25															1				1	
26																				
27															2				2	
28															2				2	
29																				
30	1										1									
31	1										1				1				1	
32													1		5				5	
33	1				1				1		3				4				4	
34															4				4	
35	1				1						2		1				2	1	2	1
36					1						1		1	1	5			1	5	1
37	2				2	1		1	1	1	5	3	2		5	3			5	3
38	2								1	2	3	2	1	1	3	2	2	1	5	3
39	3		1		1						5				8	4	1	1	9	5
40	2	3	2		1			2			5	5	1	3	2	3	2	1	4	4
41	2	1			4			3		1	6	5	5	4	2	1	1	4	3	5
42	1					2	2	1	1		4	3	3	3	1	3	1	4	2	7
43	1	1	1			1					2	2	6	4		5	1	2	1	7
44	2	2						2			2	4	2	1	2	4	4	2	6	6
45					3	1	1			1	4	2	1	1		1	1	2	1	3
46	3	1	1		1	3		1			5	5	5		1	1		4	1	5
47	2	1			1	1		1	1		4	2	2	1	1	1	1	2	2	3
48	1	1					1	3			2	4		2		2	2	1	2	3
49	1							1			1	1	1	1			1		1	
50		3			1	1		1			1	5				1				1
51	1	1				1					1	2		1		3				3
52	1	4									1	4		1		4				4
53	2	2						1			2	3				1	2	1	2	2
54	1	1						1			1	2	1			2				2
55		2										2		1						
56	1	3									1	3		1		3		1		4
57		2				1						3				1				1
58						1						1				1				1
59		1										1		1						
60		1		1		2						4	1							
61		1										1								
62		2				1						3				1				1
63		4										4		1		1				1
64		1										1		2						
65																				
66																				
67																1				1
Total	32	38	5	1	17	16	4	17	5	5	63	77	34	30	49	49	21	28	70	77

the numbers of daughters and of sons from each mating for each facet count. Under each generation the first vertical column gives the mating in the direct line. The others are sib-matings. The few exceptions to this rule are noted in the pedigree charts of tables 2 and 3.

When the mating is not of brother with sister, the fact is indicated by the presence of two catalog numbers in the third horizontal column, the upper one giving the origin of the female parent and the lower of the male parent. When more than a single male or female is included in a bottle that fact is indicated by the presence of more than one facet count under "female or male parent."

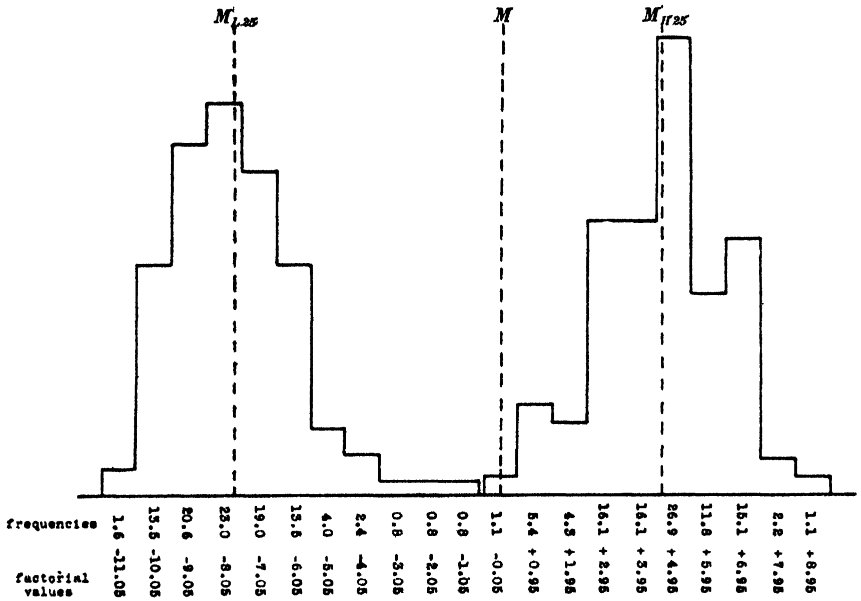


FIGURE 9.—Low-selection and high-selection males of the 25th generation arranged in classes each of which has a range equal to ten percent of its mean facet value. 126 individuals in low-selection line and 93 in high. M_{L25} = mean of low-selection line = −7 76 factorial units. M_{H25} = mean of high-selection line = +4 60 factorial units. M = mean of the unselected males = 0 00.

With very few exceptions only one of the two eyes was observed, and in the few cases in which facet counts of both eyes were made, in every instance only the eye first observed is included in the record.

Parents of direct-line offspring

The values of the parents in each generation of the direct line are given in table 8 for the low line and table 9 for the high line. The vertical

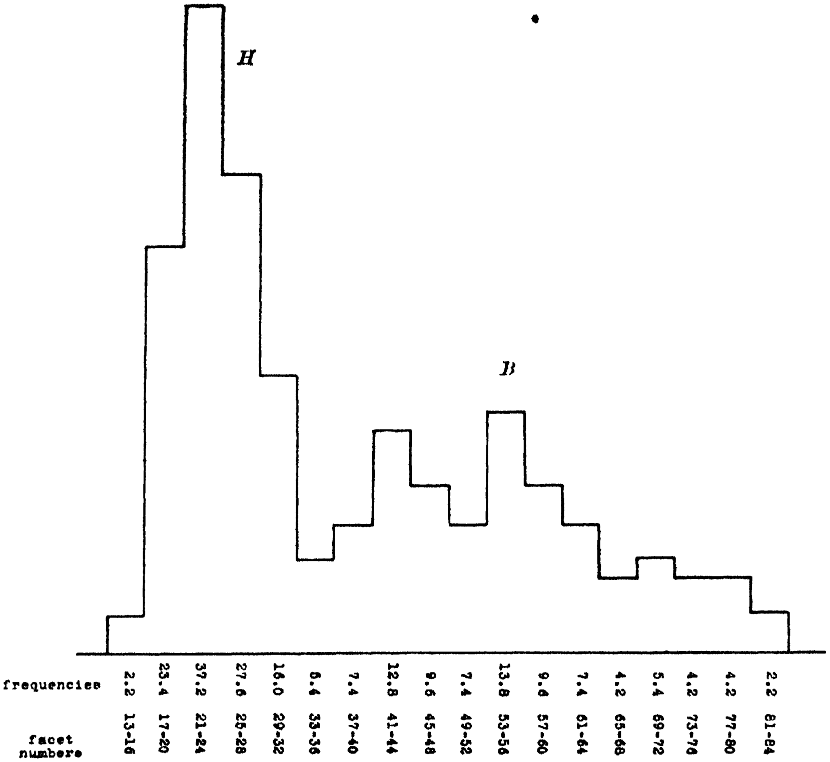


FIGURE 10.—Daughters of heterozygous female (from ultra-bar × bar) × bar male arranged in classes each with a range equal to four facets. 188 individuals. From experiment number 740. The heterozygotes (H) and bars (B) are not as well brought out as in the other method of tabulation.

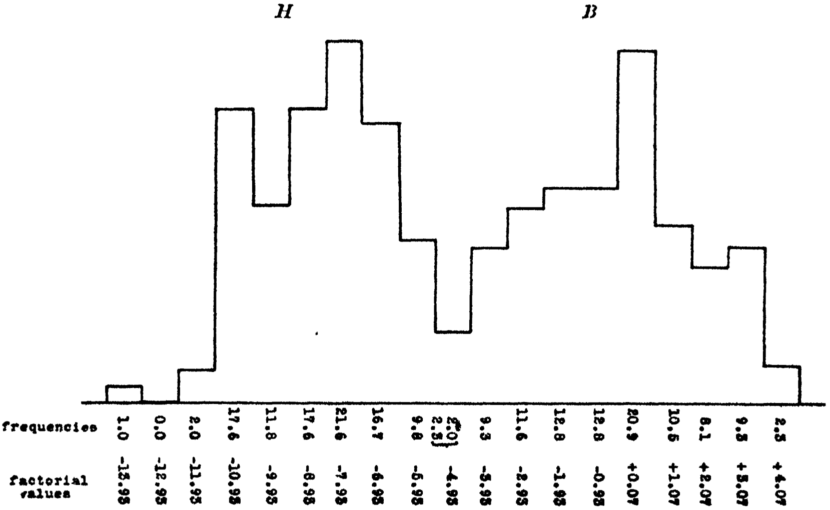


FIGURE 11.—Daughters of heterozygous female (from ultra bar × bar) × bar male arranged in classes each with a range equal to 10 percent of its mean facet value. 188 individuals. From experiment number 740. The two expected groups, heterozygotes (H) and bars (B) are well shown and agree in value with the controls.

TABLE 8

White bar selection. Low line. Direct. Parents.

GENERATION	CATALOG NUMBER	FEMALES		MALES		AVERAGE OF FACTORIAL UNITS
		Facets	Factorial units	Facets	Factorial units	
P	133–7					
1	138.3, 5	35, 30	−5.43	67, 54	− 5.40	− 5.41
2	144.4	34	−4.93	33	−11.38	− 8.15
3	146.2	44	−2.33	48	− 7.65	− 4.99
4	150.3	33	−5.26	39	− 9.67	− 7.46
5	154.m		−1.03		− 6.03	− 3.53
6	162.3	43	−2.55	51	− 7.05	− 4.80
7	168.1	37	−4.05	43	− 8.67	− 6.36
8	174.2	31, 39	−4.74	44	− 8.45	− 6.59
9	180.3	57	+0.15	51	− 7.05	− 3.45
10	192.4	50	−1.13	64	− 4.80	− 2.96
11	198.1	39	−3.55	41	− 9.17	− 6.36
12	204.1	37	−4.05	42	− 8.92	− 6.48
13	212.1	33	−5.26	41	− 9.17	− 7.21
14	218.2	37	−4.05	41	− 9.17	− 6.61
15	224.1	38	−3.80	40	− 9.42	− 6.61
16	230.2	32	−5.60	35	−10.72	− 8.16
17	236.1	30	−6.26	31	−12.05	− 9.15
18	365.3	35	−4.60	39	− 9.67	− 7.13
19	369.1	39	−3.55	44	− 8.45	− 6.00
20	375.1	30	−6.26	33	−11.38	− 8.82
21	379.2	40	−3.30	39	− 9.67	− 6.48
22	383.2	33	−5.26	43	− 8.67	− 6.96
23	387.1	40	−3.30	41	− 9.17	− 6.23
24	391.2	30	−6.26	36	−10.42	− 8.34
25	395.5b	23	−8.68	41	− 9.17	− 8.92
26	411.5	43	−2.55	45	− 8.25	− 5.40
27	417.4	41	−3.05	44	− 8.45	− 5.75
28	421.1	39	−3.55	43	− 8.67	− 6.11
29	425.5	30	−6.26	36	−10.42	− 8.34
30	429.1	32	−5.60	37	−10.17	− 7.88
31	433.3	33	−5.26	38	− 9.92	− 7.59
32	437.1	36, 36	−4.30	48	− 7.65	− 5.97
33	443.3	36	−4.30	37	−10.17	− 7.23
34	447.2	34	−4.93	38	− 9.92	− 7.42
35	451.4	34	−4.93	37	−10.17	− 7.55
36	455.2	32 33, 33	−5.26	39	− 9.67	− 7.46
37	459.3	34, 34	−4.93	42	− 8.92	− 6.92
38	463.1	33	−5.26	40	− 9.42	− 7.34
39	467.1	35	−4.60	36	−10.42	− 7.51
40	471.4	25	−7.93	34	−11.05	− 9.49
41	475.2	39	−3.55	42	− 8.92	− 6.23
42	479.x	34	−4.93	36	−10.42	− 7.67

Table 9

White bar selection. High line. Direct. Parents.

GENERATION	CATALOG NUMBER	FEMALES		MALES		AVERAGE OF FACTORIAL UNITS	DIFFERENCE BETWEEN HIGH AND LOW LINES
		Facets	Factorial units	Facets	Factorial units		
P	137.2						
1	141.2	110	+ 6.80	243	+ 8.55	+ 7.67	13.08
2	142.3	124	+ 7.99	195	+ 6.37	+ 7.18	15.33
3	148.6	144	+ 9.46	162	+ 4.54	+ 7.00	11.99
4	152.4	122	+ 7.84	153	+ 3.95	+ 5.89	13.35
5	156.1	182	+11.81	245	+ 8.61	+10.21	13.74
6	160.3	114	+ 7.16	215	+ 7.33	+ 7.24	12.04
7	166.2	135	+ 8.82	172	+ 5.13	+ 6.97	13.33
8	172.2	139	+ 9.10	187	+ 5.95	+ 7.52	14.11
9	176.5	127	+ 8.22	167	+ 4.83	+ 6.52	9.97
10	182.1	154	+10.14	273	+ 9.72	+ 9.93	12.89
11	194.1	112	+ 6.98	218	+ 7.47	+ 7.22	13.58
12	200.1	124	+ 7.99	182	+ 5.69	+ 6.84	13.32
13	206.1	127	+ 8.22	292	+10.40	+ 9.31	16.52
14	214.2	131	+ 8.53	202	+ 6.71	+ 7.62	14.23
15	222.2	131	+ 8.53	208	+ 7.00	+ 7.76	14.37
16	228.5	121	+ 7.76	168	+ 4.89	+ 6.32	14.48
17	240.3	193	+12.39	233	+ 8.12	+10.25	19.40
18	367.4	83	+ 3.88	104	+ 0.10	+ 1.99	9.12
19	373.1	128	+ 8.30	199	+ 6.57	+ 7.43	13.43
20	377.1, 2	91, 95 96, 97	+ 5.26	129, 139	+ 2.62	+ 3.94	12.76
21	381.3, 5	173, 119	+ 9.45	204, 113	+ 5.99	+ 7.72	14.20
22	385.5	275	+15.91	mass	+ 4.93	+10.42	17.38
23							
24	393.2	205	+12.97	192	+ 6.21	+ 9.59	17.93
25	399.1	204	+12.93	264	+ 9.39	+11.16	20.08
26	409.1	194	+12.44	226	+ 7.82	+10.13	15.53
27	415.1	174, 178	+11.48	247	+ 8.71	+10.09	15.84
28	419.1	192	+12.33	222	+ 7.64	+ 9.98	16.09
29	423.3	315	+17.26	237	+ 8.30	+12.78	21.12
30	427.1	247	+14.83	258	+ 9.15	+11.99	19.87
31	431.2	223	+13.81	216	+ 7.37	+10.59	18.18
32	435.4	195	+12.49	191	+ 6.16	+ 9.32	15.29
33	441.2	142, 148 154, 204	+10.66	157	+ 4.21	+ 7.43	14.66
34	445.4	178	+11.60	285, 297	+10.36	+10.98	18.40
35	449.5	152, 153	+10.03	200	+ 6.62	+ 8.32	15.87
36	453.2	167	+10.95	256	+ 9.07	+10.01	17.47
37	457.3	162	+10.66	212	+ 7.19	+ 8.92	15.84
38	461.1	155	+10.20	236	+ 8.25	+ 9.22	16.56
39	465.1	204	+12.93	230	+ 7.99	+10.46	17.97
40	469.2	167	+10.95	214	+ 7.28	+ 9.11	18.60
41	473.1	158	+10.40	200	+ 6.62	+ 8.51	14.74
42	477x.1	162	+10.66	208	+ 7.00	+ 8.83	16.50
Average							15.35±0.28

column at the left gives the generations, then come the catalog numbers of the matings, the facet counts of the parents and their equivalents on the factorial scale, and finally in the last column the averages of the factorial values of the two parents. It will be noted that the average of the parents is consistently low in the low line and high in the high line. The lower values of the males as compared with the females, is not due to any difference in the degree of selection in the two sexes but to the change in sexual dimorphism during the early generations (ZELENY 1921 b). The averages of the two sexes in each line serve to correct these values and to make them comparable in the two lines. To the high-line table are added these corrected differences between the parents of the high and low lines. On the average the parents of the high line are 15.35 ± 0.28 10-percent factorial units higher than those of the low line.

Parents of all offspring

The values of the parents including all matings are given in tables 10 and 11. These give the generations and catalog numbers of the different matings in each generation, the number of daughters and of sons resulting from each mating, the totals for each mating and for all the matings of each generation. Then come the facet values of female and male parents and their equivalents on the factorial scale, followed by the averages of the parents of individual matings, and finally by the weighted averages of the parents of all the matings in each generation on the basis of numbers of offspring. As in the direct line, due allowance should be made for the change in sexual dimorphism, but as in that case the averages of the two sexes serve to adjust the values. To the high-line table are added the corrected differences between the two lines. The high-line average is 14.66 ± 0.24 ten-percent factorial units above that of the low line.

According to the experimental procedure, it was to be expected that the divergence between the high-selected parents and the low-selected parents in any generation should be greater in the direct line than in the sib-matings. The vicissitudes of the experiment, as previously mentioned, frequently interfered with the attempt to use the highest (or lowest) pair as the direct line. However, the wider divergence comes there in 27 cases as opposed to only 14 cases for "all matings."

Offspring of direct-line matings

Tables 12 to 15 give for the offspring of the direct lines the numbers of offspring, the ranges in facet numbers and their equivalents on the fac-

TABLE 10

Values of selected pairs for all matings in each generation. Low line

GENERATION	MATING NUMBER	NUMBER OF OFFSPRING				FACETS		FACTORIAL VALUES			Weighted average of generation
		♀	♂	Total	Grand total	♀	♂	♀	♂	Average	
1	138.3	12	22	34		55	67	− 4.60	− 4.47	− 4.47	
	138.5	99	102	201	235	50	54	− 6.26	6.47	6.36	− 6.09
2	144.4	73	63	136		54	35	− 4.93	−11.58	− 8.16	
	144.1	6	7	13		38	74	− 5.80	−11.05	− 7.42	
	144.2	82	67	149		4	55	− 4.95	− 5.80	− 5.36	
	144.3	17	21	38	336	56	57	− 4.30	− 5.97	− 5.14	− 6.55
3	146.2	5	4	9		44	48	− 2.33	− 7.65	− 4.99	
	146.4	59	41	100		40	41	− 1.93	− 9.17	− 5.55	
	146.5	36	36	72	181	44	48	− 2.33	− 7.65	− 4.99	− 5.30
4	150.3	30	41	71	71	35	59	− 5.25	− 9.67	− 7.46	− 7.46
5	154 m	13	15	28	28	mass	mass	− 1.03	− 6.93	− 3.53	− 3.53
6	162.3	20	22	42		43	51	− 2.55	− 7.05	− 4.80	
	162.1	6	6	12		37	57	− 4.05	−10.17	− 7.11	
	162.2	21	11	32	86	41	43	− 3.05	− 8.67	− 5.86	− 5.52
7	168.1	47	32	79	79	37	45	− 4.05	− 8.67	− 6.36	− 6.36
8	174.2	73	54	127		51 59	44	− 4.74	− 8.45	− 6.60	
	174.1	24	34	58	185	42	58	− 2.80	− 5.80	− 4.30	− 5.88
9	180.3	32	27	59		57	51	+ 0.15	− 7.05	− 3.45	
	180.1	11	4	15		45	47	− 2.13	− 7.55	− 4.99	

9	180.2	15	14	29		54	51	− 0.35	− 7.05	− 3.70	
	180.4	31	32	63		50	54	− 1.13	− 6.47	− 3.80	
	180.5	31	39	70	236	48	54	− 1.53	− 6.47	− 4.00	− 3.84
10	192.4	72	66	138		50	64	− 1.13	− 4.80	− 2.96	
	192.1	16	19	35		57	51	+ 0.15	− 7.05	− 3.45	
	192.2	21	17	38		45, 46	47	− 2.03	− 7.85	− 4.94	
	192.3	13	9	22	233	58	56	+ 0.32	− 6.13	− 2.90	− 3.35
11	198.1	48	46	94		39	41	− 3.55	− 9.17	− 6.36	
	198.2	24	14	38	132	43	44	− 2.55	− 8.45	− 5.50	− 6.11
12	204.1	89	100	189		37	42	− 4.05	− 8.92	− 6.48	
	204.2	10	9	19	208	44	47	− 2.33	− 7.85	− 5.09	− 6.35
13	212.1	5	7	12		33	41	− 5.26	− 9.17	− 7.22	
	212.2	5	8	13		41	43	− 3.05	− 8.67	− 5.86	
	212.3	99	101	200	225	35	42	− 4.60	− 8.92	− 6.76	− 6.73
14	218.2	2	3	5		37	41	− 4.05	− 9.17	− 6.61	
	218.1	6	3	9		37	53	− 4.05	− 6.65	− 5.35	
	218.3	4	1	5		39	47	− 3.55	− 7.85	− 5.70	
	218.4	34	27	61		38	40	− 3.80	− 9.42	− 6.61	
	218.5	50	71	121	201	34	39	− 4.93	− 9.67	− 7.30	− 6.95
15	224.1	43	62	105		38	40	− 3.80	− 9.42	− 6.61	
	224.2	49	61	110		36	42	− 4.30	− 8.92	− 6.61	
	224.3	13	16	29	244	38	38	− 3.80	− 9.92	− 6.86	− 6.64
16	230.2	47	61	108		32	35	− 5.60	−10.72	− 8.16	
	230.1	24	41	65	173	35	35	− 4.60	−10.72	− 7.66	− 7.97
17	236.1	8	8	16		30	31	− 6.26	−12.05	− 9.16	
	236.2	36	44	80		34	34	− 4.93	−11.05	− 7.99	
	236.3	56	52	108	204	34, 34	37	− 4.93	−10.17	− 7.55	− 7.85

TABLE 10 (continued)

Values of selected pairs for all matings in each generation. Low line.

GENERATION	CATALOG NUMBER	NUMBER OF OFFSPRING				FACETS		FACTORIAL UNITS			
		♀	♂	Total	Grand total	♀	♂	♀	♂	Average	Weighted average of generation
18	365.3	33	41	74		35	39	− 4.60	− 9.67	− 7.14	
	365.1	5	9	14		32	34	− 5.60	−11.05	− 8.32	
	365.2	4	2	6		35	37	− 4.60	−10.17	− 7.38	
	365.4	24	20	44		37	41	− 4.05	− 9.17	− 6.61	
	365.5	9	10	19		38	41	− 3.80	− 9.17	− 6.48	
	365.6	6	5	11	168	39, 39	41	− 3.55	− 9.17	− 6.36	− 6.98
19	369.1	22	52	74		39	44	− 3.55	− 8.45	− 6.00	
	369.2	53	45	98		38	40	− 3.80	− 9.42	− 6.61	
	369.3	13	15	28		42	{42, 44 45}	− 2.80	− 8.45	− 5.62	
	369.4	34	25	59	259	46	46	− 1.93	− 8.05	− 4.99	− 5.96
20	375.1	9	18	27		30	33	− 6.26	−11.38	− 8.82	
	375.2	4	5	9		32	37	− 5.60	−10.17	− 7.88	
	375.3	19	14	33		37	38	− 4.05	− 9.92	− 6.98	
	375.4	28	32	60	129	39	41	− 3.55	− 9.17	− 6.36	− 7.14
21	379.2	29	51	80		40	39	− 3.30	− 9.67	− 6.48	
	379.1	38	61	99		37	37	− 4.05	−10.17	− 7.11	
	379.3	7	14	21		33	37	− 5.26	−10.17	− 7.72	
	379.4	4	10	14	214	35	36, 36	− 4.60	−10.42	− 7.51	− 6.96
22	383.2	14	21	35		33	43	− 5.26	− 8.67	− 6.96	
	383.1	4	10	14		33	37	− 5.26	−10.17	− 7.72	
	383.3	5	4	9		38	33	− 3.80	−11.38	− 7.59	
	383.4	8	14	22	80	38	38	− 3.80	− 9.92	− 6.86	− 7.14

23	387.1	50	58	108		40	41	− 3.30	− 9.17	− 6.24	
	387 2	21	21	42		43	48	− 2 55	− 7 65	− 5.10	
	387.3	2	10	12		37	44	− 4.05	− 8 45	− 6 25	
	387.4	9	11	20	182	43, 52	46, 55	− 1.83	− 7.15	− 4 49	− 5.79
24	391 2	71	62	133		30	36	− 6 26	−10.42	− 8 34	
	391.1	37	37	74		29	34	− 6 60	−11.05	− 8.82	
	391.3	10	8	18		30	36	− 6 26	−10 42	− 8.34	
	391.4	7	15	22		31	39	− 5.93	− 9 67	− 7 80	
	391.5	4	2	6	253	34	40	− 4 93	− 9 42	− 7.18	− 8.41
25	395.5b	5	12	17		23	41	− 8 68	− 9 17	− 8 92	
	395 1	10	25	35		22	29	− 9.18	−12.72	−10.95	
	395.2	40	50	90		33	38	− 5 26	− 9 92	− 7.59	
	395.3	11	8	19		33	36	− 5 26	−10 42	− 7 84	
	395.3b	13	16	29		23	42	− 8 68	− 8 92	− 8 80	
	395.4	3	3	6		29	37	− 6.60	−10 17	− 8.38	
	395.5	10	8	18		32	36	− 5.60	−10 42	− 8 01	
	395.6	3	4	7	221	21, 25	30	− 8.68	−12 38	−10.53	− 8.55
26	411.5	23	25	48		43	45	− 2.55	− 8 25	− 5 40	
	411.1	10	5	15		43	50	− 2 55	− 7 25	− 4.90	
	411 2	20	19	39		37	41	− 4.05	− 9.17	− 6 61	
	411.4	5	7	12	114	41	44	− 3 05	− 8 45	− 5 75	− 5.78
27	417.4	31	24	55		41	44	− 3.05	− 8 45	− 5 75.	
	417.1	22	12	34		37	38	− 4 05	− 9 92	− 6.98	
	417.2	7	6	13		35	42	− 4 60	− 8.92	− 6.76	
	417.3	9	14	23		39	43	− 3.55	− 8.67	− 6.11	
	417.5	23	24	47	172	38	40	− 3.80	− 9.42	− 6.61	− 6 35

TABLE 10 (continued)

Values of selected pairs for all matings in each generation. Low line.

GENERATION	CATALOG NUMBER	NUMBER OF OFFSPRING				FACETS		FACTORIAL UNITS			
		♀	♂	Total	Grand total	♀	♂	♀	♂	Average	Weighted average of generation
28	421.1	47	39	86		39	43	− 3.55	− 8.67	− 6.11	
	421.2	7	5	12	98	30	35	− 6.26	−10.72	− 8.49	− 6.40
29	425.5	25	23	48		30, 38	36	− 6.26	−10.42	− 8.34	
	425.1	15	15	30		32	39	− 5.60	− 9.67	− 7.64	
	425.2	2	2	4		35	36	− 4.60	−10.42	− 7.51	
	425.3	5	9	14		37	37	− 4.05	−10.17	− 7.11	
	425.4	4	8	12	108	33 (41)	35 (41)	− 5.26	−10.72	− 7.99	− 7.92
30	429.1	21	29	50		32	37	− 5.60	−10.17	− 7.88	
	429.2	7	8	15		38	36	− 3.80	−10.42	− 7.11	
	429.3	5	5	10	75	38	38	− 3.80	− 9.92	− 6.86	− 7.59
31	433.3	42	28	70		33	38	− 5.26	− 9.92	− 7.59	
	433.1	14	15	29		37, 37	39	− 4.05	− 9.67	− 6.86	
	433.2	28	32	60		35	37	− 4.60	−10.17	− 7.38	
	433.4	2	5	7		35	39	− 4.60	− 9.67	− 7.14	
	433.5	2	3	5	171	35	41	− 4.60	− 9.17	− 6.88	− 7.35
32	437.1	49	44	93		36, 36	48	− 4.30	− 7.65	− 5.98	
	437.2	3	10	13		31	32	− 5.93	−11.72	− 8.82	
	437.3	15	21	36		32	51	− 5.60	− 7.05	− 6.32	
	437.4	11	18	29		52, 49 51, 47	49, 47	− 1.13	− 7.65	− 4.39	
	437.5	22	18	40		34	49	− 4.05	− 7.45	− 5.75	
	437.6	6	6	12		37	47	− 4.93	− 7.85	− 6.39	
	437.7	3	0	3	226	34	48	− 4.93	− 7.65	− 6.29	− 5.98

	443.3	35	35	70		[illegible]	[illegible]				
33	443.1	4	6	10		33	35	— 5 26	—10 72	— 7 99	
	443.2	13	12	25	105	35	39	— 4.60	— 9.67	— 7.14	— 7.29
34	447.2	24	21	45		34	38	— 4.93	— 9.92	— 7.42	
	447.1	9	13	22	67	33	38	— 5.26	— 9.92	— 7.59	— 7.48
35	451.4	26	19	45		34	37	— 4.93	—10.17	— 7.55	
	451 1	14	22	36		38	40	— 3.80	— 9.42	— 6.61	
	451.2	18	25	43		38	40	— 3.80	— 9.42	— 6.61	
	451.3	4	6	10	134	41	39	— 3.05	— 9.67	— 6.36	— 6.91
36	455.2	47	48	95		{32, 33 33}	39	— 5 26	— 9.67	— 7.46	
	455.1	10	11	21		38	39, 39	— 3.80	— 9.67	— 6 74	
	455.4	34	33	67	183	31	36	— 5.93	—10 42	— 8.18	— 7.64
37	459.3	32	38	70		34, 34	42	— 4 93	— 8.92	— 6.92	
	459.1	5	1	6		33	31	— 5.26	—12.05	— 8.66	
	459 11	17	16	33		35	35	— 4 60	—10 72	— 7.66	
	459.2	4	17	21		34	39	— 4 93	— 9.67	— 7.30	
	459.4	5	5	10	140	33	41	— 5 26	— 9 17	— 7.22	— 7.25
38	463.1	34	30	64	64	33	40	— 5.26	— 9.42	— 7.34	— 7.34
39	467 1	49	49	98		35	36	— 4.60	—10 42	— 7.51	
	467.2	21	28	49	147	38	43, 43	— 3 80	— 8.67	— 6.24	— 7.09
40	471.4	7	6	13		25	34	— 7 93	—11.05	— 9.49	
	471.1	26	36	62		39	39	— 3.55	— 9 67	— 6.61	
	471.2	26	16	42		38	41	— 3 80	— 9 17	— 6.48	
	471 3	18	11	29		39	39	— 3 55	— 9.67	— 6.61	
	471 5	8	8	16	162	35	36	— 4 60	—10.42	— 7.51	— 6.90
41	475.2	19	31	50		39	42	— 3 55	— 8.92	— 6.24	
	475.1	12	19	31	81	37	38	— 4 05	— 9.92	— 6 98	— 6.52
42	479 x	32	24	56	56	34	36	— 4 93	—10.42	— 7.68	— 7.68

TABLE 11

Values of selected pairs for all matings in each generation. High line.

GENERATION	CATALOG NUMBER	NUMBER OF OFFSPRING				FACETS		FACTORIAL UNITS				
		♀	♂	Total	Grand total	♀	♂	♀	♂	Average	Weighted average of generation	Difference between high and low lines
1	141.2	96	92	188		110	243	+ 6.80	+ 8.55	+ 7.68		
	141.3	18	11	29		110	234	+ 6.80	+ 8.17	+ 7.48		
	141.5	93	106	199	416	109	213	+ 6.71	+ 7.24	+ 6.98	+ 7.33	13.42
2	142.3	43	39	82		124	195	+ 7.99	+ 6.37	+ 7.18		
	142.1	21	22	43		104	204	+ 6.22	+ 6.81	+ 6.52		
	142.2	89	62	151		81	105	+ 3.63	+ 0.20	+ 1.92		
	142.5	11	4	15	291	134	201	+ 8.75	+ 6.66	+ 7.70	+ 4.38	10.93
3	148.6	51	50	101		144	162	+ 9.46	+ 4.54	+ 7.00		
	148.2	22	29	51		139	197	+ 9.10	+ 6.47	+ 7.78		
	148.3	13	10	23		126	176	+ 8.15	+ 5.36	+ 6.76		
	148.4	3	3	6		143	205	+ 9.39	+ 6.85	+ 8.12		
	148.5	29	34	63	244	127	157	+ 8.22	+ 4.21	+ 6.22	+ 6.97	12.27
4	152.4	40	45	85		122	153	+ 7.84	+ 3.95	+ 5.90		
	152.1	1	1	2		144	181	+ 9.46	+ 5.63	+ 7.54		
	152.2	21	29	50		132	163	+ 8.61	+ 4.60	+ 6.60		
	152.3	29	30	59		129	161	+ 8.38	+ 4.48	+ 6.43		
	152.5	32	29	61		126	147	+ 8.15	+ 3.55	+ 5.85		
	152.7	25	13	38	295	102	147	+ 6.02	+ 3.55	+ 4.78	+ 5.98	13.44
5	156.1	89	78	167		182	245	+11.81	+ 8.63	+10.22		
	156.2	19	20	39		155	179	+10.20	+ 5.53	+ 7.86		
	156.3	28	33	61		140	177	+ 9.18	+ 5.42	+ 7.30		
	156.4	1	5	6		146	178	+ 9.60	+ 5.48	+ 7.54		
	156.5	22	33	55	328	139	167	+ 9.10	+ 4.83	+ 6.96	+ 8.80	12.33

6	160.3	27	18	45		114	215	+ 7 16	+ 7.33	+ 7 24		
	160.1	8	21	29		163	234	+10 72	+ 8 17	+ 9.44		
	160.2	35	43	78		111	206	+ 6 89	+ 6 90	+ 6 90		
	160 4	6	7	13		112	175	+ 6 98	+ 5 30	+ 6 14		
	160.5	7	4	11	176	98	472	+ 5 62	+15 18	+10 40	+ 7 57	13.09
7	166.2	22	21	43		135	172	+ 8 82	+ 5.13	+ 6 98		
	166 1	21	44	65		133	180	+ 8 68	+ 5 58	+ 7.13		
	166 3	19	10	29		154	162	+10 14	+ 4 54	+ 7 34		
	166 4	26	17	43		127	163	+ 8 22	+ 4 60	+ 6 41		
	166.6	3	4	7	187	127	163	+ 8 22	+ 4 60	+ 6.41	+ 6.94	13.30
8	172 2	48	43	91		139	187	+ 9 10	+ 5 95	+ 7 52		
	172 1	8	6	14	105	144	183	+ 9 46	+ 5 74	+ 7 60	+ 7.53	13.41
9	176 5	57	51	108		127	167	+ 8 22	+ 4 83	+ 6 52		
	176 1	15	16	31		103, 103	123	+ 6 12	+ 1.80	+ 3 96		
	176 2	21	28	49		105	163	+ 6 32	+ 4 60	+ 5 46		
	176 3	19	20	39		94, 95	114	+ 5 23	+ 1 04	+ 3 14		
	176 3x	4	3	7		95	184	+ 5 29	+ 5 79	+ 5 54		
	176 4	11	8	19		137	156	+ 8 96	+ 4 15	+ 6 56		
	176 6	6	2	8	261	123	239	+ 7 92	+ 8 38	+ 8 15	+ 5 54	9.38
10	182.1	66	69	135		154	273	+10 14	+ 9 72	+ 9.93		
	182.2	9	11	20		143	174	+ 9 39	+ 5 24	+ 7 32		
	182 3	13	21	34	189	172	252, 257	+11.25	+ 9 01	+10.13	+ 9.69	13.04
11	194.1	19	22	41		112	218	+ 6 98	+ 7 47	+ 7 22		
	194 2	14	17	31		125	175	+ 8 07	+ 5.30	+ 6 68		
	194 3	13	13	26		132	232	+ 8.61	+ 8 08	+ 8.34		
	194.4	24	19	43		124	223	+ 7.99	+ 7 69	+ 7 84		
	194.5	34	47	81		121	207	+ 7.76	+ 6.95	+ 7.36		
	194.6	31	36	67	289	118	307	+ 7.52	+10.89	+ 9.20	+ 7.85	13.96

TABLE 11 (continued)

Values of selected pairs for all matings in each generation. High line.

GENERATION	CATALOG NUMBER	NUMBER OF OFFSPRING				FACETS		FACTORIAL UNITS				
		♀	♂	Total	Grand total	♀	♂	♀	♂	Average	Weighted average of generation	Difference between high and low lines
12	200.1	44	40	84		124	182	+ 7.99	+ 5.69	+ 6.84		
	200.2	8	9	17	101	105	156	+ 6.32	+ 4.15	+ 5.24	+ 6.57	12.92
13	206.1	85	87	172	172	127	292	+ 8.22	+10.40	+ 9.31	+ 9.31	16.04
14	214.2	27	19	46		143	202	+ 9.39	+ 6.71	+ 8.05		
	214.1	44	46	90	136	131	202	+ 8.53	+ 6.71	+ 7.62	+ 7.77	14.72
15	222.2	57	61	118		131	208	+ 8.53	+ 7.00	+ 7.76		
	222.1	3	2	5	123	144	233	+ 9.46	+ 8.12	+ 8.79	+ 7.80	14.44
16	228.5	48	68	116		121	168	+ 7.76	+ 4.89	+ 6.32		
	228.1	22	15	37		107	118	+ 6.52	+ 1.40	+ 3.96		
	228.2	28	37	65		128	169	+ 8.30	+ 4.95	+ 6.62		
	228.3	15	9	24		124	146	+ 7.99	+ 3.48	+ 5.74		
	228.4	32	29	71	303	126	168	+ 8.15	+ 4.89	+ 6.52	+ 6.31	14.28
17	240.3	31	34	65		193	233	+12.39	+ 8.12	+10.26		
	234.1	11	9	20		115, 118	158	+ 7.38	+ 4.28	+ 5.83		
	234.2	9	15	24		124	167	+ 7.99	+ 4.83	+ 6.41		
	240.1	4	2	6		229	278	+14.07	+ 9.90	+11.98		
	240.2	5	4	9	124	146	212	+ 9.60	+ 7.19	+ 8.40	+ 8.75	16.60
18	367.4	22	21	43	43	83	104	+ 3.88	+ 0.10	+ 1.99	+ 1.99	8.97
19	373.1	12	18	30	30	128	199	+ 8.30	+ 6.57	+ 7.44	+ 7.44	13.40
20	377.1	56	51	107		91, 95	129	+ 5.07	+ 2.26	+ 3.66		
	377.2	57	38	95		96, 97	139	+ 5.45	+ 2.98	+ 4.22		
	377.3	31	24	55		108	131	+ 6.62	+ 2.41	+ 4.52		
	377.4	3	5	8	265	109	149	+ 6.71	+ 3.68	+ 5.20	+ 4.09	11.23

21	381.3 381.5	49 24	26 14	75 38		173 119	204 173	+11.30 + 7.61	+ 6.81 + 5.18	+ 9.06 + 6.40		
	381.1	19	28	47		142	160	+ 9.32	+ 4.42	+ 6.87		
	381.2	12	3	15		158	184	+10.40	+ 5.79	+ 8.10		
	381.4	11	8	19	194	131	179	+ 8.53	+ 5.53	+ 7.03	+ 7.74	14.70
22	385.5	7	4	11		275	mass	+15.91	+ 4.93	+10.42		
	385.1	1	0	1		194	346	+12.44	+12.08	+12.26		
	385.2	35	28	63		142	232	+ 9.32	+ 8.08	+ 8.70		
	385.3	17	11	28		137	228	+ 8.96	+ 7.91	+ 8.44		
	385.4	26	22	48	151	107	189	+ 6.52	+ 6.05	+ 6.28	+ 8.03	15.17
23	389.6	43	65	108		(138,143, 156)	211	+ 9.58	+ 7.14	+ 8.36		
	389.1	11	17	28		113	154	+ 7.07	+ 4.02	+ 5.54		
	389.2	3	2	5		131	186	+ 8.53	+ 5.90	+ 7.22		
	389.3	11	10	21		125	158	+ 8.07	+ 4.28	+ 6.18		
	389.4	7	13	20		141	219	+ 9.25	+ 7.51	+ 8.38		
	389.5	4	2	6	188	145	197	+ 9.53	+ 6.47	+ 8.00	+ 7.66	13.45
24	393.2	29	15	44		205	192	+12.97	+ 6.21	+ 9.59		
	393.1	26	21	47		119	146	+ 7.61	+ 3.48	+ 5.54		
	393.11	13	18	31		122, 115	144	+ 7.56	+ 3.34	+ 5.45		
	393.3	23	21	44		121	194	+ 7.76	+ 6.32	+ 7.04		
	393.4	6	8	14		124	195	+ 7.99	+ 6.37	+ 7.18		
	393.5	5	4	9		131	198	+ 8.53	+ 6.52	+ 7.52		
	393.6	1	3	4	193	116	201	+ 7.34	+ 6.66	+ 7.00	+ 7.03	15.44
25	399.1	56	38	94		204	264	+12.93	+ 9.39	+11.16		
	399.2	13	8	21		162	254	+10.66	+ 8.99	+ 9.82		
	399.3	13	19	32		149	202	+ 9.80	+ 6.71	+ 8.26		
	399.4	13	12	25		144	238	+ 9.46	+ 8.34	+ 8.90		
	399.5	8	7	15		130	204	+ 8.45	+ 6.81	+ 7.63		
	399.6	11	9	20	207	121	178	+ 7.76	+ 5.48	+ 6.62	+ 9.61	18.16

TABLE tin

Valu for all in each ution.

GENERATION	CATALOG NUMBER	NUMBER OF OFFSPRING				FACETS		FACTORIAL UNITS				
		♀	♂	Total	Grand total	♀	♂	♀	♂	Average	Weighted average of generation	Difference between high and low lines
26	409.1	12	2	14		194	226	+12 44	+ 7 82	+10.13		
	409.2	24	24	48		212	208	+13 31	+ 7.00	+10.16		
	409.3	19	8	27		178	194	+11.60	+ 6.32	+ 8.96		
	409.4	6	8	14		197	235	+12 59	+ 8 21	+10 40		
	409.5	13	5	18	121	167	197	+10 95	+ 6 47	+ 8.71	+ 9 70	15.48
27	415 1	21	7	28		174, 178	247	+11 48	+ 8.71	+10 10		
	415.2	9	5	14		{194,212} {208}	223	+12.96	+ 7.69	+10.32		
	415 3	0	6	6		196	197	+12 54	+ 6 47	+ 9 50		
*	415.4	0	2	2	50	m	m	+10 99	+ 5 44	+ 8.22	+10.01	16.36
28	419.1	48	17	65		192	222	+12 33	+ 7.64	+ 9.98		
	419.2	5	0	5		221	219	+13 72	+ 7.51	+10 62		
	419.3	3	0	3		242	219	+14 63	+ 7 51	+11.07		
	419.4	1	2	3	76	207	209	+13 07	+ 7 04	+10 06	+10 07	16.47
29	423.3	20	17	37		315	237	+17 26	+ 8 30	+12 78		
	423.1	19	11	30		163	188	+10 72	+ 6 00	+ 8.36		
	423.2	9	6	15	82	152	178	+10.00	+ 5 48	+ 7 74	+10.24	18.16
30	427.1	13	9	22		247	258	+14 83	+ 9 15	+11 99		
	427.2	12	8	20		171	200	+11.19	+ 6.62	+ 8.90		
	427.3	20	14	34		216	329	+13 49	+11 58	+12.54		
	427 4	4	1	5	81	267	276	+15 62	+ 9 82	+12.72	+11 50	19.09

31	431.2	25	17	42	134	223	216	+13.81	+ 7.37	+10.59	+10.59	17.94
	431.1	36	24	60		212, 221	234	+13.51	+ 8.17	+10.84		
	431.3	19	13	32		216	203	+13.49	+ 6.76	+10.12		
32	435.4	31	7	38	97	195	191	+12.49	+ 6.16	+ 9.32	+ 8.59	14.57
	435.2	5	2	7		171	203	+11.19	+ 6.76	+ 8.98		
	435.3	10	2	12		178	156	+11.60	+ 4.15	+ 7.88		
	435.5	19	10	29		178	198	+11.60	+ 6.52	+ 9.06		
	435.6	6	5	11		102	164	+ 6.02	+ 4.66	+ 5.34		
33	441.2	26	27	53	149	142, 148 154, 204	157	+10.66	+ 4.21	+ 7.44	+ 9.33	16.62
	441.1	67	29	96		195	236	+12.49	+ 8.25	+10.37		
34	445.4	12	12	24	185	178	285, 297	+11.60	+10.36	+10.98	+ 8.68	16.16
	445.1	34	27	61		162, 154 168, 178	163	+10.86	+ 4.60	+ 7.73		
	445.2	10	6	16		149	158	+ 9.80	+ 4.28	+ 7.04		
	445.3	14	17	31		157, 161	197, 199	+10.47	+ 6.52	+ 8.50		
	455.5	4	5	9		159	190,220 239	+10.47	+ 7.39	+ 8.93		
	445.6	0	3	3		184	197	+11.91	+ 6.47	+ 9.19		
	445.7	25	16	41		194	196	+12.44	+ 6.42	+ 9.43		
35	449.5	14	8	22	123	152, 153	200	+10.03	+ 6.62	+ 8.32	+ 7.21	14.12
	449.1	15	6	21		143, 145 142	189, 198	+ 9.42	+ 6.29	+ 7.86		
	449.2	9	16	25		85	119	+ 4.13	+ 1.49	+ 2.81		
	449.4	18	20	38		174	167	+11.36	+ 4.83	+ 8.10		
	449.6	9	8	17		185, 188 199	202, 202	+12.26	+ 6.71	+ 9.48		

TABLE 11 (continued)

Values of selected pairs for all matings in each generation. High line.

GENERATION	CATALOG NUMBER	NUMBER OF OFFSPRING				FACETS		FACTORIAL UNITS				
		♀	♂	Total	Grand total	♀	♂	♀	♂	Average	Weighted average of generation	Difference between high and low lines
36	453.2 453.1 453.3 453.4 453.5 453.6	30 2 7 6 25 3	31 2 8 7 26 7	61 4 15 13 51 10	154	167 164 158 202 167 163	256 197 198 298 177 174, 178	+10 95 +10.78 +10.40 +12.83 +10 95 +10.72	+ 9 07 + 6.47 + 6.52 +10 60 + 5.42 +11 48	+10.01 + 8 62 + 8 46 +11.72 + 8.18 +11 10	+ 9 43	17 07
37	457 3 457.2 457.4 457 6	9 7 16 9	5 2 26 6	14 9 42 15	80	162 167 138, 142 148	212 240 198, 208 208, 211	+10 66 +10 95 + 9 18 + 9 74	+ 7 19 + 8.43 + 6 76 + 7.06	+ 8 92 + 9 69 + 7.97 + 8 40	+ 8.41	15 66
38	461.1 461.2 461.3	17 10 3	11 10 9	28 20 12	60	155 147 157, 179	236 191, 234 {203,220 237}	+10 20 + 9.67 +11 01	+ 8.25 + 7.21 + 7.56	+ 9.22 + 8.44 + 9 28	+ 8.97	16.31
39	465.1 465.2	10 28	23 32	33 60	93	204 150	230 194	+12 93 + 9.87	+ 7.99 + 6.32	+10.46 + 8 10	+ 8.94	16.03
40	469.2 469.1 469.3	21 7 41	35 11 58	56 18 99	173	167 140, 167 162, 167	214 163, 167 245	+10 95 +10 07 +10 80	+ 7.28 + 4.71 + 8.63	+ 9 12 + 7.39 + 9.72	+ 9.28	16.18
41	473.1 473.2	14 4	28 4	42 8	50	158 170	200 204	+10.40 +11.13	+ 6 62 + 6.81	+ 8 51 + 8.92	+ 8.58	15 10
42	477.x	6	12	18	18	162	208	+10 66	+ 7.00	+ 8.83	+ 8.83	16.51
											Average	14.66±0 24

TABLE 12

White bar selection. Low line. Direct. Offspring. Females.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	133-7	488	21	134	−9.68	+8.75	0.00±0.10	3.12±0.07
1	138.3, 5	111	32	71	−5.60	+2.36	−2.07±0.12	1.94±0.08
2	144.4	73	32	119	−5.60	+7.61	+1.29±0.20	2.57±0.14
3	146.2	5	33	45	−5.26	−2.13	−3.13±0.35	1.17±0.25
4	150.3	30	31	85	−5.93	+4.13	−1.03±0.32	2.62±0.23
5	154 m	13	37	66	−4.05	+1.64	−1.93±0.28	1.52±0.20
6	162.3	20	35	67	−4.60	+1.78	−1.63±0.27	1.82±0.19
7	168.1	47	31	58	−5.93	+0.32	−2.38±0.17	1.79±0.12
8	174.2	73	25	84	−7.93	+4.01	+0.08±0.16	1.96±0.11
9	180.3	32	40	63	−3.30	+1.15	−0.77±0.13	1.06±0.09
10	192.4	72	39	78	−3.55	+3.26	−0.83±0.14	1.71±0.10
11	198.1	48	33	73	−5.26	+2.63	−1.08±0.18	1.85±0.13
12	204.1	88	24	84	−8.26	+4.01	−1.98±0.12	1.71±0.19
13	212.1	5	37	44	−4.05	−2.33	−3.13±0.23	0.75±0.16
14	218.2	2	38	38	−3.80	−3.80	−3.80	
15	224.1	43	31	58	−5.93	+0.32	−3.09±0.13	1.29±0.09
16	230.2	47	30	59	−6.26	+0.49	−2.76±0.17	1.69±0.12
17	236.1	8	32	41	−5.60	−3.05	−4.31±0.21	0.86±0.15
18	365.3	33	26	64	−7.60	+1.32	−3.20±0.22	1.85±0.15
19	369.1	22	30	45	−6.26	−2.13	−4.11±0.17	1.15±0.12
20	375.1	9	33	41	−5.26	−3.05	−4.04±0.17	0.74±0.12
21	379.2	29	30	49	−6.26	−1.33	−3.62±0.14	1.11±0.10
22	383.2	14	37	49	−4.05	−1.33	−2.72±0.16	0.87±0.11
23	387.1	50	29	52	−6.60	−0.73	−3.55±0.14	1.46±0.10
24	391.2	71	20	55	−10.18	−0.18	−4.87±0.18	2.26±0.13
25	395.5b	5	37	48	−4.05	−1.53	−2.73±0.23	0.75±0.16
26	411.5	23	34	47	−4.93	−1.73	−3.06±0.11	0.79±0.08
27	417.4	31	29	47	−6.60	−1.73	−3.70±0.17	1.41±0.12
28	421.1	47	30	53	−6.26	−0.53	−3.59±0.12	1.24±0.09
29	425.5	25	32	47	−5.60	−1.73	−3.81±0.16	1.18±0.11
30	429.1	21	33	48	−5.26	−1.53	−3.69±0.13	0.87±0.09
31	433.3	42	30	57	−6.26	+0.15	−2.36±0.12	1.20±0.09
32	437.1	48	35	75	−4.60	+2.88	−1.89±0.15	1.57±0.11
33	443.3	35	25	46	−7.93	−1.93	−4.56±0.14	1.26±0.10
34	447.2	24	30	43	−6.26	−2.55	−4.01±0.15	1.08±0.10
35	451.4	26	29	42	−6.60	−2.80	−4.31±0.15	1.14±0.11
36	455.2	47	19	54	−10.68	−0.35	−3.57±0.16	1.63±0.11
37	459.3	32	30	56	−6.26	−0.01	−2.71±0.18	1.56±0.13
38	463.1	34	32	60	−5.60	+0.65	−2.64±0.14	1.25±0.10
39	467.1	49	25	47	−7.93	−1.73	−4.40±0.14	1.39±0.10
40	471.4	7	36	44	−4.30	−2.33	−3.36±0.19	0.73±0.13
41	475.2	19	28	42	−6.93	−2.80	−4.14±0.17	1.10±0.12
42	479.x	32	35	51	−4.60	−0.93	−2.84±0.12	1.01±0.08

TABLE 13

White bar selection. Low line. Direct. Offspring. Males.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	133-7	441	35	314	−10.72	+11.11	0.00±0.13	3.91±0.09
1	138.3,5	124	33	124	−11.38	+ 1.87	−4.85±0.16	2.66±0.11
2	144.4	62	47	134	− 7.85	+ 2.63	−2.58±0.23	2.69±0.16
3	146.2	4	35	59	−10.72	− 5.63	−8.30±0.77	2.28±0.54
4	150.3	41	41	78	− 9.17	− 2.86	−6.03±0.18	1.70±0.13
5	154.m	15	37	82	−10.17	− 2.36	−5.85±0.42	2.40±0.29
6	162.3	22	43	83	− 8.67	− 2.24	−5.14±0.29	2.02±0.20
7	168.1	32	44	97	− 8.45	− 0.61	−5.24±0.22	1.82±0.15
8	174.2	54	46	86	− 8 05	− 1.86	−5.12±0.14	1.50±0.10
9	180.3	27	43	68	− 8.67	− 4.19	−6.20±0.16	1.21±0.11
10	192.4	66	37	97	−10.17	− 0.61	−6.47±0.16	1.88±0.11
11	198.1	46	41	97	− 9.17	− 0.61	−5.03±0.24	2.44±0.17
12	204.1	100	29	86	−12.72	− 1.86	−6.61±0.12	1.78±0.09
13	212.1	7	41	53	− 9.17	− 6.65	−8.05±0 19	0.75±0.13
14	218.2	3	40	51	− 9.42	− 7.05	−8.38±0.37	0.94±0.26
15	224.1	62	34	78	−11.05	− 2.86	−7.95±0.15	1.79±0.11
16	230.2	61	31	66	−12 05	− 4.48	−8.67±0.18	2.05±0.13
17	236 1	8	34	44	−11.05	− 8.45	−9.30±0.23	0.97±0.16
18	365.3	41	32	90	−11.72	− 1.38	−7.81±0.23	2.14±0.16
19	369.1	52	33	63	−11.38	− 4.97	−9.45±0.10	1.10±0.07
20	375.1	18	34	47	−11.05	− 7.85	−9.61±0.11	0.69±0.08
21	379.2	51	29	63	−12.72	− 4.97	−9.15±0.15	1.54±0.10
22	383.2	21	41	61	− 9.17	− 5.30	−7.76±0.17	1.16±0.12
23	387.1	58	30	67	−12 38	− 4.34	−8.29±0.13	1.47 ±0.09
24	391.2	62	29	78	−12.72	− 2.86	−7.82±0.19	2.27±0.14
25	395.5b	12	41	58	− 9.17	− 5.80	−7.80±0.18	0.93±0.13
26	411.5	25	35	54	−10.72	− 6.47	−8.57±0.17	1.27±0.12
27	417.4	24	35	57	−10.72	− 5.97	−8.68±0.20	1.47±0.14
28	421.1	39	35	74	−10.72	− 3.36	−8.36±0.19	1.73±0.13
29	425.5	23	33	58	−11.05	− 5.80	−9.18±0.19	1.36±0.13
30	429.1	29	35	55	−10.72	− 6.30	−8.77±0.15	1.17±0.10
31	433.3	28	39	62	− 9.67	− 5.13	−7.41±0.14	1.08±0.10
32	437.1	44	37	98	−10.17	− 0.50	−7.00±0.22	2.12±0.15
33	443.3	35	29	64	−12.72	− 4.80	−8.71±0.17	1.49±0.12
34	447.2	21	34	52	−11.05	− 6.85	−9.10±0.16	1.09±0.11
35	451.4	19	36	64	−10.42	− 4.80	−7.68±0.24	1.53±0.17
36	455.2	48	37	58	−11.05	− 5.80	−8.76±0.11	1.16±0.08
37	459.3	38	37	64	− 9.42	− 4.80	−6.73±0.14	1.32±0.10
38	463.1	30	36	64	−10.42	− 4.80	−8.05±0.18	1.46±0.13
39	467.1	49	37	67	−10.17	− 4.34	−7.99±0.15	1.56±0.11
40	471.4	6	37	48	−10.17	− 7.65	−9.22±0.19	0.69±0.13
41	475.2	31	32	51	−11.72	− 7.05	−9.31±0.15	1.22±0.10
42	479.x	24	41	58	− 9.17	− 5.80	−7.59±0.15	1.08±0.10

TABLE 14

White bar selection. High line. Direct. Offspring. Females.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	137.2	488	21	134	− 9.68	+ 8.75	0.00±0.10	3.12±0.07
1	141.2	96	38	144	− 3.80	+ 9.46	+ 3.03±0.22	3.26±0.16
2	142.3	43	76	166	+ 3.01	+10.89	+ 7.09±0.15	1.46±0.11
3	148.6	51	84	146	+ 4.01	+ 9.60	+ 6.87±0.12	1.24±0.08
4	152.4	40	84	182	+ 4.01	+11.81	+ 8.22±0.18	1.70±0.13
5	156.1	88	63	242	+ 1.15	+14.63	+ 8.22±0.19	2.59±0.13
6	160.3	27	91	155	+ 4.85	+10.20	+ 7.22±0.19	1.43±0.13
7	166.2	22	72	144	+ 2.50	+ 9.46	+ 6.30±0.32	2.21±0.22
8	172.2	48	70	137	+ 2.21	+ 8.96	+ 4.99±0.17	1.76±0.12
9	176.5	57	64	181	+ 1.32	+11.75	+ 7.84±0.23	2.52±0.16
10	182.1	60	88	167	+ 4.51	+10.95	+ 7.39±0.13	1.47±0.09
11	194.1	19	101	134	+ 5.92	+ 8.75	+ 7.28±0.16	1.06±0.11
12	200.1	44	90	154	+ 4.74	+10.14	+ 7.27±0.14	1.37±0.10
13	206.1	85	79	143	+ 3.38	+ 9.39	+ 6.81±0.09	1.22±0.06
14	214.2	27	93	144	+ 5.07	+ 9.46	+ 7.26±0.15	1.12±0.10
15	222.2	57	93	156	+ 5.07	+10.27	+ 6.86±0.11	1.18±0.07
16	228.5	48	82	248	+ 3.76	+14.87	+ 8.17±0.31	3.22±0.22
17	240.3	31	83	139	+ 3.88	+ 9.10	+ 6.65±0.18	1.47±0.13
18	367.4	22	68	141	+ 1.93	+ 9.25	+ 6.07±0.28	1.93±0.20
19	373.1	12	91	132	+ 4.85	+ 8.61	+ 6.32±0.23	1.17±0.16
20	377.1,2	113	72	179	+ 2.50	+11.65	+ 6.72±0.11	1.80±0.08
21	381.3,5	73	86	275	+ 4.26	+15.91	+ 7.47±0.16	2.01±0.11
22	385.5	7	94	205	+ 5.18	+12.97	+ 8.78±0.62	2.45±0.44
23								
24	393.2	29	66	206	+ 1.64	+13.02	+ 8.07±0.36	2.91±0.25
25	399.1	56	57	247	+ 0.15	+14.83	+ 8.89±0.25	2.77±0.18
26	409.1	12	108	217	+ 6.62	+13.54	+10.99±0.44	2.25±0.31
27	415.1	21	117	242	+ 7.43	+14.63	+11.02±0.32	2.19±0.23
28	419.1	48	83	315	+ 3.88	+17.26	+11.65±0.27	2.75±0.19
29	423.3	20	141	262	+ 9.25	+15.43	+12.37±0.28	1.87±0.20
30	427.1	13	116	249	+ 7.34	+14.91	+11.45±0.39	2.10±0.28
31	431.2	25	78	202	+ 3.26	+12.83	+ 9.43±0.32	2.35±0.22
32	435.4	31	65	204	+ 1.49	+12.93	+ 9.04±0.30	2.48±0.21
33	441.2	26	105	207	+ 6.32	+13.07	+ 9.72±0.22	1.66±0.16
34	445.4	12	116	170	+ 7.34	+11.13	+ 9.15±0.22	1.11±0.15
35	449.5	14	96	167	+ 5.40	+10.95	+ 9.21±0.31	1.73±0.22
36	453.2	29	94	248	+ 5.18	+14.87	+ 9.17±0.27	2.14±0.19
37	457.3	9	115	188	+ 7.25	+12.12	+10.74±0.32	1.41±0.22
38	461.1	17	102	204	+ 6.02	+12.93	+ 9.48±0.29	1.75±0.20
39	465.1	10	103	167	+ 6.12	+10.95	+ 8.77±0.36	1.68±0.25
40	469.2	21	101	184	+ 5.92	+11.91	+ 9.36±0.23	1.55±0.16
41	473.1	14	137	173	+ 8.96	+11.30	+10.28±0.14	0.77±0.10
42	477x.1	9	101	142	+ 5.92	+ 9.32	+ 8.07±0.24	1.05±0.17

TABLE 15

White bar selection. High line. Direct. Offspring. Males.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	137.2	441	35	314	−10.72	+11.11	0.00±0.13	3.91±0.09
1	141.2	92	44	237	− 8.45	+ 8.30	−0.35±0.24	3.45±0.17
2	142.3	39	111	197	+ 0.77	+ 6.47	+3.33±0.16	1.45±0.11
3	148.6	50	92	184	− 1.16	+ 5.79	+2.29±0.15	1.53±0.10
4	152.4	45	76	245	− 3.11	+ 8.63	+3.71±0.21	2.06±0.15
5	156.1	78	118	472	+ 1.40	+15.18	+6.99±0.23	3.01±0.16
6	160.3	18	104	229	+ 0.10	+ 7.95	+3.23±0.28	1.79±0.20
7	166.2	21	97	187	− 0.61	+ 5.95	+4.00±0.24	1.59±0.17
8	172.2	43	85	239	− 1.99	+ 8.38	+3.04±0.22	2.14±0.16
9	176.5	51	128	348	+ 2.18	+12.14	+6.46±0.22	2.32+0.15
10	182.1	69	107	316	+ 0.40	+11.05	+5.44±0.19	2.40±0.14
11	194.1	22	122	212	+ 1.72	+ 7.19	+4.54±0.19	1.37±0.13
12	200.1	40	129	384	+ 2.26	+13.12	+6.43±0.24	2.22±0.17
13	206.1	87	99	220	− 0.40	+ 7.56	+3.89±0.13	1.73±0.09
14	214.2	19	117	208	+ 1.31	+ 7.00	+4.16±0.23	1.51±0.16
15	222.2	61	101	206	− 0.20	+ 6.90	+3.15±0.14	1.63±0.10
16	228.5	68	95	278	− 0.83	+ 9.90	+4.21±0.22	2.63±0.15
17	240.3	34	104	238	+ 0.10	+ 8.34	+3.86±0.25	2.17±0.18
18	367.4	21	87	323	− 1.74	+11.40	+4.24±0.46	3.17±0.33
19	373.1	18	97	199	− 0.61	+ 6.57	+2.51±0.29	1.80±0.20
20	377.1,2	90	94	215	− 0.94	+ 7.33	+3.05±0.14	2.05±0.10
21	381.3,5	40	112	238	+ 0.86	+ 8.34	+4.93±0.19	1.75±0.13
22	385.5	4	129	192	+ 2.26	+ 6.21	+3.45±0.51	1.50±0.36
23								
24	393.2	15	97	264	− 0.61	+ 9.39	+3.82±0.52	2.96±0.36
25	399.1	38	108	244	+ 0.50	+ 8.59	+4.95±0.22	2.04±0.16
26	409.1	2	128	247	+ 2.18	+ 8.71	+5.44	
27	415.1	7	146	251	+ 3.48	+ 8.87	+7.09±0.44	1.73±0.31
28	419.1	17	152	287	+ 3.88	+10.22	+6.77±0.30	1.82±0.21
29	423.3	17	137	329	+ 2.84	+11.58	+7.71±0.35	2.13±0.25
30	427.1	9	163	284	+ 4.60	+10.11	+6.73±0.35	1.55±0.25
31	431.2	17	122	248	+ 1.72	+ 8.75	+5.60±0.32	1.97±0.23
32	435.4	7	133	182	+ 2.56	+ 5.69	+4.38±0.27	1.05±0.19
33	441.2	27	130	297	+ 2.33	+10.56	+5.69±0.29	2.23±0.20
34	445.4	12	109	320	+ 0.59	+11.31	+5.70±0.61	3.11±0.43
35	449.5	8	155	298	+ 4.08	+10.60	+7.20±0.52	2.17±0.37
36	453.2	31	138	265	+ 2.91	+ 9.43	+6.27±0.21	1.73±0.15
37	457.3	5	178	256	+ 5.48	+ 9.07	+6.75±0.35	1.17±0.25
38	461.1	10	115	230	+ 1.13	+ 7.99	+5.15±0.48	2.23±0.34
39	465.1	23	124	246	+ 1.87	+ 8.67	+4.73±0.25	1.79±0.18
40	469.2	35	134	221	+ 2.63	+ 7.60	+5.44±0.17	1.46±0.12
41	473.1	28	150	241	+ 3.75	+ 8.47	+6.09±0.19	1.46±0.13
42	477x.1	17	119	380	+ 1.49	+13.07	+5.19±0.41	2.53±0.29

torial scale and the means and standard deviations and their probable errors also in terms of the factorial scale. Table 12 includes the females and table 13 the males of the low line, and table 14 the females and table 15 the males of the high line. Mutants to full eye and to ultra-bar are omitted in these and following tables unless specifically mentioned.

It will be noted that the number of individuals is often too small for a satisfactory determination of the standard deviation. In such cases the values are usually lower than in the generations with larger numbers of individuals, probably because they usually represent the hatch of a single day and therefore have a greater environmental uniformity than those representing the hatch of several days, frequently with transfer of the parents to additional bottles. In the determination of the standard deviation values also the number of classes was often less than ten and the accuracy is somewhat lowered by that fact. It is believed, however, that no significant error is thereby introduced as far as the purposes of the present discussion are concerned.

Offspring of all matings

Tables 16 to 19 give the values for the offspring of all matings. Each table includes the generation number, catalog number, number of offspring, range in facets and in their equivalents on the factorial scale and the means and standard deviations and their probable errors also in terms of the factorial scale. Table 16 includes the females and table 17 the males of the low line, and table 18 the females and table 19 the males of the high line. The data have the advantage over those of the direct line in possessing in most generations a sufficient number of individuals for determination of the standard deviation. One would expect them, however, to contain a greater diversity in both germinal and environmental factors. A comparison of the average standard deviations, however, shows only a slight difference in this respect in favor of "all matings." Among low-line females the direct line has the greater standard deviation in 14 generations, "all matings" in 18, and 9 are even. Among low-line males the corresponding figures are 13, 23 and 6, among high-line females 17, 20 and 4, and among high males 16, 20 and 4.

Distribution of frequencies in the direct lines

The values are given in table 20 for females and table 21 for males. Each table includes at the left the class values in factorial units and their ranges in facet numbers. Then follow for each selection generation

TABLE 16

White bar selection. Low line. All matings. Offspring. Females.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	133–7	488	21	134	− 9.68	+ 8.75	0.00±0.10	3.12±0.07
1	138	111	32	71	− 5.60	+ 2.36	−2.07±0.12	1.94±0.08
2	144	178	32	119	− 5.60	+ 7.61	+0.73±0.11	2.25±0.08
3	146	100	33	65	− 5.26	+ 1.49	−1.86±0.10	1.53±0.07
4	150	30	31	85	− 5.93	+ 4.13	−1.03±0.32	2.62±0.23
5	154	13	37	66	− 4.05	+ 1.64	−1.93±0.28	1.52±0.20
6	162	47	35	67	− 4.60	+ 1.78	−1.61±0.18	1.82±0.13
7	168	47	31	58	− 5.93	+ 0.32	−2.38±0.17	1.79±0.12
8	174	97	25	84	− 7.93	+ 4.01	−0.19±0.13	1.83±0.09
9	180	119	39	83	− 3.55	+ 3.88	−0.64±0.09	1.45±0.06
10	192	122	39	84	− 3.55	+ 4.01	−0.30±0.10	1.70±0.07
11	198	72	33	76	− 5.26	+ 3.01	−0.72±0.14	1.82±0.10
12	204	98	24	84	− 8.26	+ 4.01	−1.82±0.12	1.71±0.09
13	212	109	32	68	− 5.60	+ 1.93	−2.38±0.10	1.57±0.07
14	218	96	33	58	− 5.26	+ 0.32	−2.86±0.08	1.22±0.06
15	224	105	29	58	− 6.60	+ 0.32	−3.26±0.08	1.16±0.05
16	230	71	30	59	− 6.26	+ 0.49	−2.86±0.12	1.55±0.09
17	236	100	32	66	− 5.60	+ 1.64	−1.66±0.11	1.60±0.08
18	365	81	26	64	− 7.60	+ 1.32	−2.87±0.12	1.55±0.08
19	369	122	30	58	− 6.26	+ 0.32	−2.67±0.08	1.28±0.06
20	375	60	32	54	− 5.60	− 0.35	−2.81±0.12	1.37±0.08
21	379	78	30	62	− 6.26	+ 0.99	−3.34±0.10	1.27±0.07
22	383	31	37	52	− 4.05	− 0.73	−2.54±0.11	0.91±0.08
23	387	82	29	52	− 6.60	− 0.73	−3.39±0.10	1.31±0.07
24	391	129	20	62	−10.18	+ 0.99	−4.45±0.13	2.22±0.09
25	395	95	31	62	− 5.93	+ 0.99	−2.47±0.10	1.39±0.07
26	411	58	34	67	− 4.93	+ 1.78	−2.55±0.10	1.16±0.07
27	417	92	29	62	− 6.60	+ 0.99	−3.23±0.09	1.34±0.07
28	421	54	30	53	− 6.26	− 0.53	−3.41±0.12	1.28±0.08
29	425	51	32	50	− 5.60	− 1.13	−3.24±0.11	1.14±0.08
30	429	33	33	48	− 5.26	− 1.53	−3.51±0.10	0.89±0.07
31	433	88	30	57	− 6.26	+ 0.15	−2.79±0.10	1.39±0.07
32	437	108	33	75	− 5.26	+ 2.88	−2.62±0.09	1.45±0.06
33	443	52	25	46	− 7.93	− 1.93	−4.33±0.12	1.26±0.08
34	447	33	30	44	− 6.26	− 2.33	−3.93±0.13	1.10±0.09
35	451	62	29	55	− 6.60	− 0.18	−3.62±0.13	1.47±0.09
36	455	91	19	54	−10.68	− 0.35	−3.80±0.10	1.44±0.07
37	459	63	30	56	− 6.26	− 0.01	−2.85±0.11	1.32±0.08
38	463	34	32	60	− 5.60	+ 0.65	−2.64±0.14	1.25±0.10
39	467	70	25	53	− 7.93	− 0.53	−3.86±0.13	1.56±0.09
40	471	85	30	56	− 6.26	− 0.01	−2.81±0.08	1.07±0.06
41	475	31	23	54	− 8.68	− 0.35	−3.80±0.19	1.58±0.13
42	479.x	32	35	51	− 4.60	− 0.93	−2.84±0.12	1.01±0.08

TABLE 17

White bar selection. Low line. All matings. Offspring. Males.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	133-7	441	35	314	−10.72	+11.11	0.00±0.13	3.91±0.09
1	138	124	33	124	−11.38	+ 1.87	−4.85±0.16	2.66±0.11
2	144	157	41	134	− 9.17	+ 2.63	−3.44±0.14	2.54±0.09
3	146	81	35	91	−10.72	− 1.27	−6.35±0.14	1.91±0.10
4	150	41	41	78	− 9.17	− 2.86	−6.03±0.18	1.70±0.13
5	154	15	37	82	−10.17	− 2.36	−5.85±0.42	2.40±0.29
6	162	39	36	83	−10.42	− 2.24	−5.67±0.23	2.17±0.17
7	168	32	44	97	− 8.45	− 0.61	−5.24±0.22	1.82±0.15
8	174	88	39	86	− 9.67	− 1.86	−5.47±0.12	1.68±0.09
9	180	116	23	99	−14.80	− 0.40	−6.08±0.11	1.76±0.08
10	192	111	37	101	−10.17	− 0.20	−5.44±0.14	2.22±0.10
11	198	60	41	99	− 9.17	− 0.40	−4.85±0.22	2.50±0.15
12	204	109	29	86	−12.72	− 1.86	−6.56±0.11	1.74±0.08
13	212	116	38	87	− 9.92	− 1.74	−6.62±0.12	1.89±0.08
14	218	105	32	63	−11.72	− 4.97	−8.55±0.09	1.37±0.06
15	224	139	34	111	−11.05	+ 0.77	−7.96±0.11	1.86±0.07
16	230	102	31	66	−12.05	− 4.48	−8.67±0.12	1.81±0.09
17	236	104	34	99	−11.05	− 0.40	−7.12±0.14	2.14±0.10
18	365	87	32	90	−11.72	− 1.38	−7.92±0.12	1.67±0.08
19	369	137	33	86	−11.38	− 1.86	−7.98±0.11	1.91±0.08
20	375	69	34	66	−11.05	− 4.48	−8.60±0.11	1.40±0.08
21	379	136	29	67	−12.72	− 4.34	−9.13±0.09	1.52±0.06
22	383	49	38	69	− 9.92	− 4.05	−7.74±0.12	1.21±0.08
23	387	100	30	67	−12.38	− 4.34	−8.09±0.10	1.52±0.07
24	391	124	13	78	−20.22	− 2.86	−8.34±0.14	2.23±0.10
25	395	126	35	91	−10.72	− 1.27	−7.76±0.11	1.77±0.08
26	411	56	35	83	−10.72	− 2.24	−7.41±0.16	1.75±0.11
27	417	80	32	73	−11.72	− 3.49	−8.60±0.13	1.67±0.09
28	421	44	35	74	−10.72	− 3.36	−8.23±0.17	1.68±0.12
29	425	57	33	67	−11.38	− 4.34	−8.42±0.15	1.63±0.10
30	429	42	35	55	−10.72	− 6.30	−8.74±0.11	1.08±0.08
31	433	83	32	71	−11.72	− 3.76	−7.68±0.10	1.39±0.07
32	437	117	32	98	−11.72	− 0.50	−8.05±0.11	1.79±0.08
33	443	53	29	64	−12.72	− 4.80	−8.90±0.13	1.39±0.09
34	447	34	34	52	−11.05	− 6.85	−9.05±0.12	1.00±0.08
35	451	72	32	73	−11.72	− 3.49	−7.90±0.13	1.69±0.09
36	455	92	33	58	−11.38	− 5.80	−9.06±0.08	1.17±0.06
37	459	77	37	64	−10.17	− 4.80	−7.36±0.12	1.52±0.08
38	463	30	36	64	−10.42	− 4.80	−8.05±0.18	1.46±0.13
39	467	77	35	67	−10.72	− 4.34	−8.23±0.11	1.41±0.08
40	471	77	37	56	−10.17	− 6.13	−8.37±0.08	1.03±0.06
41	475	50	32	55	−11.72	− 6.30	−9.15±0.13	1.32±0.09
42	479x	24	41	58	− 9.17	− 5.80	−7.59±0.15	1.08±0.10

TABLE 18

White bar selection. High line. All matings. Offspring. Females.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	133-7	488	21	134	− 9.68	+ 8.75	0.00±0.10	3.12±0.07
1	141	207	35	203	− 4.60	+12.88	+ 2.04±0.15	3.23±0.11
2	142	164	37	166	− 4.05	+10.89	+ 5.53±0.13	2.41±0.09
3	148	118	51	146	− 0.93	+ 9.60	+ 5.76±0.12	1.95±0.08
4	152	148	67	182	+ 1.78	+11.81	+ 7.19±0.10	1.75±0.07
5	156	158	63	242	+ 1.15	+14.63	+ 8.13±0.12	2.25±0.08
6	160	82	84	182	+ 4.01	+11.81	+ 6.92±0.12	1.62±0.08
7	166	91	44	144	− 2.33	+ 9.46	+ 5.34±0.18	2.59±0.13
8	172	56	70	137	+ 2.21	+ 8.96	+ 4.91±0.15	1.69±0.11
9	176	133	63	181	+ 1.15	+11.75	+ 6.79±0.14	2.37±0.10
10	182	82	88	167	+ 4.51	+10.95	+ 7.29±0.10	1.41±0.07
11	194	135	74	164	+ 2.76	+10.78	+ 6.63±0.09	1.53±0.06
12	200	52	88	154	+ 4.51	+10.14	+ 7.15±0.13	1.38±0.09
13	206	85	79	143	+ 3.38	+ 9.39	+ 6.81±0.09	1.22±0.06
14	214	71	93	150	+ 5.07	+ 9.87	+ 7.76±0.10	1.23±0.07
15	222	60	68	156	+ 1.93	+10.27	+ 6.69±0.12	1.41±0.09
16	228	145	72	248	+ 2.50	+14.87	+ 6.97±0.14	2.54±0.10
17	240	60	55	186	− 0.18	+12.02	+ 6.65±0.18	2.07±0.13
18	367	22	68	141	+ 1.93	+ 9.25	+ 6.07±0.28	1.93±0.20
19	373	12	91	132	+ 4.85	+ 8.61	+ 6.32±0.23	1.17±0.16
20	377	147	58	179	+ 0.32	+11.65	+ 6.62±0.10	1.83±0.07
21	381	115	86	275	+ 4.26	+15.91	+ 7.60±0.12	1.97±0.09
22	385	86	39	227	− 3.55	+13.98	+ 7.00±0.21	2.89±0.15
23	389	79	36	172	− 4.30	+11.25	+ 6.91±0.17	2.21±0.12
24	393	103	66	206	+ 1.64	+13.02	+ 7.80±0.14	2.16±0.10
25	399	114	57	247	+ 0.15	+14.83	+ 8.01±0.15	2.40±0.11
26	409	73	83	220	+ 3.88	+13.68	+ 9.81±0.17	2.20±0.12
27	415	30	87	242	+ 4.38	+14.63	+ 9.47±0.38	3.12±0.27
28	419	57	83	315	+ 3.88	+17.26	+11.53±0.24	2.70±0.17
29	423	48	102	268	+ 6.02	+15.66	+11.76±0.20	2.05±0.14
30	427	49	109	249	+ 6.71	+14.91	+10.91±0.19	1.99±0.14
31	431	80	72	397	+ 2.50	+19.56	+ 9.82±0.21	2.79±0.15
32	435	71	65	228	+ 1.49	+14.03	+ 9.62±0.20	2.41±0.14
33	441	93	82	248	+ 3.76	+14.87	+ 9.24±0.16	2.25±0.11
34	445	99	72	230	+ 2.50	+14.11	+ 8.33±0.15	2.17±0.10
35	449	66	71	202	+ 2.36	+12.83	+ 8.27±0.21	2.50±0.15
36	453	72	68	248	+ 1.93	+14.87	+ 8.35±0.18	2.29±0.13
37	457	42	108	188	+ 6.62	+12.12	+10.09±0.13	1.22±0.09
38	461	30	102	204	+ 6.02	+12.93	+ 9.47±0.19	1.56±0.14
39	465	38	103	180	+ 6.12	+11.70	+ 9.31±0.17	1.60±0.12
40	469	69	100	184	+ 5.82	+11.91	+ 8.91±0.11	1.40±0.08
41	473	18	137	180	+ 8.96	+11.70	+10.40±0.13	0.82±0.09
42	477x	9	101	142	+ 5.92	+ 9.32	+ 8.07±0.24	1.05±0.17

TABLE 19

White bar selection. High line. All matings. Offspring. Males.

GENERATION	CATALOG NUMBER	NUMBER OF INDIVIDUALS	FACETS		FACTORIAL UNITS			
			Range		Range		Mean	Standard deviation
			Low	High	Low	High		
P	133–7	441	35	314	−10.72	+11.11	0.00±0.13	3.91±0.09
1	141	209	44	237	− 8.45	+ 8.30	−1.32±0.17	3.62±0.12
2	142	127	52	227	− 6.85	+ 7.86	+1.19±0.19	3.16±0.13
3	148	126	42	212	− 8.92	+ 7.19	+1.09±0.15	2.49±0.11
4	152	147	76	245	− 3.11	+ 8.63	+2.79±0.11	2.00±0.08
5	156	169	91	472	− 1.27	+15.18	+5.48±0.15	2.80±0.10
6	160	93	103	242	0.00	+ 8.51	+3.24±0.12	1.67±0.08
7	166	96	77	238	− 2.99	+ 8.34	+2.92±0.16	2.28±0.11
8	172	49	85	239	− 1.99	+ 8.38	+2.66±0.22	2.28±0.15
9	176	128	87	348	− 1.74	+12.14	+4.02±0.17	2.91±0.12
10	182	101	102	316	− 0.10	+11.05	+4.81±0.16	2.38±0.11
11	194	154	86	238	− 1.86	+ 8.34	+3.80±0.12	2.18±0.08
12	200	49	112	384	+ 0.86	+13.12	+5.77±0.24	2.50±0.17
13	206	87	99	220	− 0.40	+ 7.56	+3.89±0.13	1.73±0.09
14	214	65	117	289	+ 1.31	+10.29	+5.06±0.13	1.54±0.09
15	222	63	93	206	− 1.05	+ 6.90	+3.01±0.15	1.76±0.11
16	228	158	91	287	− 1.27	+10.22	+3.65±0.13	2.35±0.09
17	240	64	104	238	+ 0.10	+ 8.34	+3.72±0.16	1.85±0.11
18	367	21	87	323	− 1.74	+11.40	+4.24±0.46	3.17±0.33
19	373	18	97	199	− 0.61	+ 6.57	+2.51±0.29	1.80±0.20
20	377	118	94	215	− 0.94	+ 7.33	+3.16±0.12	1.91±0.08
21	381	79	104	346	+ 0.10	+12.08	+5.18±0.16	2.12±0.11
22	385	65	99	258	− 0.40	+ 9.15	+4 60±0.16	1.95±0.11
23	389	109	87	294	− 1.74	+10.47	+4.12±0.14	2.23±0.10
24	393	90	97	288	− 0.61	+10.25	+4.65±0.15	2.11±0.11
25	399	93	106	244	+ 0.30	+ 8.59	+4.60±0.13	1.81±0.09
26	409	47	108	278	+ 0.50	+ 9.90	+5.50±0.23	2.29±0.16
27	415	20	112	251	+ 0.86	+ 8.87	+5.50±0.36	2.42±0.26
28	419	19	134	287	+ 2.63	+10.22	+6.48±0.30	1.95±0.21
29	423	34	137	329	+ 2.84	+11.58	+6.86±0.24	2.05±0.17
30	427	32	142	285	+ 3.20	+10.15	+6.11±0.20	1.72±0.14
31	431	54	119	408	+ 1.49	+13.72	+5.49±0.20	2.23±0.15
32	435	26	133	263	+ 2.56	+ 9.35	+5.10±0.23	1.77±0.16
33	441	56	103	297	0 00	+10.56	+4.90±0.19	2.17±0.14
34	445	86	104	365	+ 0.10	+12.62	+4.72±0.18	2.49±0.13
35	449	56	94	298	− 0.94	+10.60	+4.83±0.18	2.01±0.13
36	453	81	88	265	− 1.61	+ 9.43	+4.81±0.17	2.29±0.12
37	457	39	142	256	+ 3.20	+ 9.07	+6.41±0.17	1.57±0.12
38	461	29	115	230	+ 1.13	+ 7.99	+5.36±0.23	1.87±0.16
39	465	55	124	246	+ 1.87	+ 8.67	+5.22±0.16	1.73±0.11
40	469	104	114	378	+ 1.04	+12.96	+5.11±0.12	1.89±0.09
41	473	32	150	241	+ 3.75	+ 8.47	+6.14±0.16	1.38±0.12
42	477x	17	119	380	+ 1.49	+13.02	+5.19±0.41	2.53±0.29

TABLE 20

Distribution of frequencies of factorial values. Low and high selection. Females. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	P	FREQUENCIES 1		2		3		4		5		6		7		8		9		10		11	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1										1												
+20.07	397–438																							
+19.07	359–396																							
+18.07	325–358																							
+17.07	294–324																							
+16.07	266–293	1																						
+15.07	241–265											1												
+14.07	218–240																							
+13.07	197–217											1												
+12.07	178–196									1		9								1				
+11.07	161–177					1				1		7								5		1		
+10.07	146–160					2		1		7		10		3						10		4		
+ 9.07	132–145	1		4		3		2		9		12		2		5		2		11		5		3
+ 8.07	119–131			5	1	7		13		9		7		4		3		2		9		19		4
+ 7.07	108–118	7		4		13		14		5		15		7		2		4		4		13		6
+ 6.07	98–107	11		11		14		13		6		15		9		4		10		9		11		6
+ 5.07	89– 97	27		10	5	2		7		1		6		2		3		10		1		6		
+ 4.07	81– 88	25		11	12			1	1	1		2				1	1	7		3		1		
+ 3.07	73– 80	29		7	4	1			2			2				3	5	10			5		1	
+ 2.07	66– 72	51	3	11	12				3		1		1			1	11	3		3	3		3	
+ 1.07	60– 65	55	7	8	10				3			1	2				8		2	1	6		4	
+ 0.07	54– 59	54	14	11	13				4		1		3		8		25		11		10		11	
– 0.93	49– 53	58	21	3	4				3		1		1		10		12		13		15		7	
– 1.93	44– 48	54	12	7	7		2		3		4		6		6		6		2		24		13	
– 2.93	40– 43	47	9	2	2		1		5		5		4		8		2		4		7		4	
– 3.93	36– 39	40	26	2	2		1		3		1		2		8						2		2	
– 4.93	33– 35	15	4				1		2				1		5		2						3	
– 5.93	30– 32	9	3		1				1						2									
– 6.93	27– 29	3																						
– 7.93	24– 26	1															1							
– 8.93	22– 23																							
– 9.93	20– 21	1																						
–10.93	18– 19																							
Totals		490	99	96	73	43	5	51	30	40	13	89	20	27	47	22	73	48	32	57	72	60	48	19

TABLE 20 (continued)

Distribution of frequencies of factorial values. Low and high selection. Females. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																						
		P	12		13		14		15		16		17		18		19		20		21		22	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1	1																					
+20.07	397–438																							
+19.07	359–396																							
+18.07	325–358																							
+17.07	294–324																							
+16.07	266–293	1																				1		
+15.07	241–265											1												
+14.07	218–240											2												
+13.07	197–217											2												1
+12.07	178–196											6												
+11.07	161–177											3										2		
+10.07	146–160			3						1		1										3		2
+ 9.07	132–145	1		4		5		2		2		4		3		2		1		2		13		1
+ 8.07	119–131			12		20		11		16		6		7		4				5		20		
+ 7.07	108–118	7		10		22		7		10		4		6		3		4		10		11		2
+ 6.07	98–107	11		10		29		4		21		4		7		5		3		19		10		
+ 5.07	89– 97	27		5		5		3		7		9		5		3		4		11		7		1
+ 4.07	81– 88	25	1			3						6		3		2				8		6		
+ 3.07	73– 80	29				1										2								
+ 2.07	66– 72	51														1				1				
+ 1.07	60– 65	55	2												1									
+ 0.07	54– 59	54	12						1		5				2									
− 0.93	49– 53	58	17						4		6				1						1		1	
− 1.93	44– 48	54	23		1				8		10				6		2				3		4	
− 2.93	40– 43	47	19		2				10		7		1		7		3		2		7		6	
− 3.93	36– 39	40	8		2		2		15		13		4		12		10		4		13		3	
− 4.93	33– 35	15	4						4		2		2				3		3		3			
− 5.93	30– 32	9	1						1		4		1		2		4				2			
− 6.93	27– 29	3													1									
− 7.93	24– 26	1	1												1									
− 8.93	22– 23																							
− 9.93	20– 21	1																						
−10.93	18– 19																							
Totals		490	89	44	5	85	2	27	43	57	47	48	8	31	33	22	22	12	9	56	29	73	14	7

Table 20 (continued)

Distribution of frequencies of factorial values. Low and high selection. Females. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																				
		P	23		24		25		26		27		28		29		30		31		32	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1																			1	
+20.07	397–438																					
+19.07	359–396																					
+18.07	325–358																					
+17.07	294–324													1								
+16.07	266–293	1												3								
+15.07	241–265							1				1		4		3		1				
+14.07	218–240											3		2		4		2				
+13.07	197–217					3		3		4		1		8		3		1		2		1
+12.07	178–196					1		7		3		4		6		1		1		3		4
+11.07	161–177					2		5		1		3		11		5		3		5		4
+10.07	146–160					5		7		1		1		3		3		4		2		4
+ 9.07	132–145	1				1		8				6		5		1				2		7
+ 8.07	119–131					3		9		1		1		1						7		4
+ 7.07	108–118	7				2		5		2		1		2				1		2		2
+ 6.07	98–107	11				7		3												1		3
+ 5.07	89– 97	27				2		6						1								1
+ 4.07	81– 88	25				2		1						1								
+ 3.07	73– 80	29																		1	1	
+ 2.07	66– 72	51				1																
+ 1.07	60– 65	55																			2	1
+ 0.07	54– 59	54			2			1											2		4	
– 0.93	49– 53	58	2		2								4						6		9	
– 1.93	44– 48	54	10		6		2		5		7		2		4		2		16		17	
– 2.93	40– 43	47	16		8		2		11		7		13		4		5		10		5	
– 3.93	36– 39	40	6		14		1		6		8		19		11		10		7		9	
– 4.93	33– 35	15	10		13				1		6		5		3		4				1	
– 5.93	30– 32	9	5		6						1		4		3				1			
– 6.93	27– 29	3	1		10						2											
– 7.93	24– 26	1			5																	
– 8.93	22– 23				4																	
– 9.93	20– 21	1			1																	
–10.93	18– 19																					
Totals		490	50	0	71	27	5	56	23	12	31	21	47	48	25	20	21	13	42	25	49	31

TABLE 20 (continued)

Distribution of frequencies of factorial values. Low and high selection. Females. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	P	FREQUENCIES																			
			33		34		35		36		37		38		39		40		41		42	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1								1												
+20.07	397–438																					
+19.07	359–396																					
+18.07	325–358																					
+17.07	294–324																					
+16.07	266–293	1																				
+15.07	241–265									1												
+14.07	218–240																					
+13.07	197–217			1										1								
+12.07	178–196			3						1		2		1				1				
+11.07	161–177			3		1		4		6		5		2		2		4		6		
+10.07	146–160			8		4		3		5		1		4		1		5		5		
+ 9.07	132–145	1		5		3		2		4				5		3		6		3		4
+ 8.07	119–131			3		3		3		6				1		2		1				2
+ 7.07	108–118	7		2		1		1		3		1		2				3				2
+ 6.07	98–107	11		1						1				1		2		1				1
+ 5.07	89– 97	27						1		2												
+ 4.07	81– 88	25																				
+ 3.07	73– 80	29																				
+ 2.07	66– 72	51																				
+ 1.07	60– 65	55											1									
+ 0.07	54– 59	54							1		2		1									
– 0.93	49– 53	58							2		5		1								2	
– 1.93	44– 48	54	1						6		8		10		4		1				11	
– 2.93	40– 43	47	4		9		7		13		6		15		5		2		6		8	
– 3.93	36– 39	40	14		8		8		13		7		4		21		4		6		10	
– 4.93	33– 35	15	8		3		6		11		2		1		8				5		1	
– 5.93	30– 32	9	5		4		4				2		1		6				1			
– 6.93	27– 29	3	2				1								4				1			
– 7.93	24– 26	1	1												1							
– 8.93	22– 23																					
– 9.93	20– 21	1																				
–10.93	18– 19								1													
Totals		490	35	26	24	12	26	14	47	30	32	9	34	17	49	10	7	21	19	14	32	9

TABLE 21

Distribution of frequencies of factorial values. Low and high selection. Males. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																						
		P	1		2		3		4		5		6		7		8		9		10		11	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye																								
+14.95	439–484											1												
+13.95	397–438											3												
+12.95	359–396											2												
+11.95	325–358											2								1				
+10.95	294–324	1																		2		3		
+ 9.95	266–293											3								2		1		
+ 8.95	241–265	3								1		10								7		2		
+ 7.95	218–240	6		1								11		1				1		5		6		
+ 6.95	197–217	15		2		1				2		14								6		9		3
+ 5.95	178–196	16		2		3		1		4		5				4		4		9		13		2
+ 4.95	161–177	26		4		4		2		11		11		4		4		9		9		14		6
+ 3.95	146–160	19		2		7		10		8		8		2		7		6		5		8		6
+ 2.95	132–145	34		7	2	15		9		6		4		3		4		4		4		6		4
+ 1.95	119–131	35		8	3	5		13		8		3		7		1		8		1		3		1
+ 0.95	108–118	33		10	6	4		10		4		1						7				3		
– 0.05	98–107	50		15	4			3						1				2				1		
– 1.05	89– 97	55		7	3			2							1	1		1			1		3	
– 2.05	81– 88	37		13	13						2		1		3		2	1			2		3	
– 3.05	73– 80	29		4	8				3	1	1		6		1		3				1		8	
– 4.05	66– 72	23	3	7	7				7		2		2		6		19		2		7		11	
– 5.05	60– 65	22	2	3	8				5		1		5		4		10		6		9		5	
– 6.05	54– 59	18	4	3	4		2		11		3		2		11		9		10		8		2	
– 7.05	49– 53	8	1	1	2				7		3		3		2		7		5		16		3	
– 8.05	44– 48	7	2	3	2		0		4				1		4		4		3		16		5	
– 9.05	40– 43	2	5						4		2		2						1		5		6	
–10.05	36– 39	1	3				1				1										1			
–11.05	33– 35	1	2				1																	
–12.05	30– 32																							
–13.05	27– 29																							
–14.05	24– 26																							
–15.05	22– 23																							
–16.05	20– 21																							
–17.05	18– 19				1																			
Total		441	22	92	63	39	4	50	41	45	15	78	22	18	32	21	54	43	27	51	66	69	46	22

TABLE 21 (continued)

Distribution of frequencies of factorial values. Low and high selection. Males. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																						
		P	12		13		14		15		16		17		18		19		20		21		22	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye																								
+14.95	439–484																							
+13.95	397–438																							
+12.95	359–396			1																				
+11.95	325–358			1																				
+10.95	294–324	1														1								
+ 9.95	266–293			1								1												
+ 8.95	241–265	3		2								2				1								
+ 7.95	218–240	6		6		1						5		3		2						4		
+ 6.95	197–217	15		7		8		2		1		10		1		3		1		4		5		1
+ 5.95	178–196	16		11		7		2		5		7		5						5		5		
+ 4.95	161–177	26		3		14		2		7		5		4		1				3		9		
+ 3.95	146–160	19		5		21		8		13		7		4		2		4		8		10		
+ 2.95	132–145	34		2		19		3		13		12		9		5		5		5		5		2
+ 1.95	119–131	35		1		10		1		12		8		3		3		4		10				1
+ 0.95	108–118	33				6		1		8		6		3		1		1				2		
– 0.05	98–107	50				1				2		4		2		1		2		2				
– 1.05	89– 97	55										1			1			1		2				
– 2.05	81– 88	37	1												1	1								
– 3.05	73– 80	29	3						1						1									
– 4.05	66– 72	23	9						2		1													
– 5.05	60– 65	22	15						3		3				2		1				1		1	
– 6.05	54– 59	18	18						7		7				3						2		3	
– 7.05	49– 53	8	24		2		1		9		9				3		1				3		3	
– 8.05	44– 48	7	16		3		0		17		8		2		16		8		1		12		8	
– 9.05	40– 43	2	13		2		2		11		9		3		7		11		7		10		6	
–10.05	36– 39	1							8		12		2		6		27		9		17			
–11.05	33– 35	1							4		7		1				4		1		3			
–12.05	30– 32										5				1						2			
–13.05	27– 29		1																		1			
–14.05	24– 26																							
–15.05	22– 23																							
–16.05	20– 21																							
–17.05	18– 19																							
Total		441	100	40	7	87	3	19	62	61	61	68	8	34	41	21	52	18	18	39	51	40	21	4

TABLE 21 (continued)

Distribution of frequencies of factorial values. Low and high selection. Males. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																				
		P	23		24		25		26		27		28		29		30		31		32	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye																						
+14.95	439–484																					
+13.95	397–438																					
+12.95	359–396																					
+11.95	325–358															1						
+10.95	294–324	1																				
+ 9.95	266–293													1		3		1				
+ 8.95	241–265	3				1		1		1		2		3		3				1		
+ 7.95	218–240	6				1		2				2		3		2		2		2		
+ 6.95	197–217	15				1		8				0		2		2		1		4		
+ 5.95	178–196	16				2		6				2		3		5		3		3		1
+ 4.95	161–177	26				2		6		0				3				2		1		3
+ 3.95	146–160	19				1		5				1		2						3		1
+ 2.95	132–145	34				2		6								1				2		2
+ 1.95	119–131	35				1		1		1										1		
+ 0.95	108–118	33				1		3														
– 0.05	98–107	50				2															1	
– 1.05	89– 97	55				1															1	
– 2.05	81– 88	37																				
– 3.05	73– 80	29			3								1								1	
– 4.05	66– 72	23	1		2																2	
– 5.05	60– 65	22	3		7								2						1		1	
– 6.05	54– 59	18	1		7		1		1		3		3		1		2		5		9	
– 7.05	49– 53	8	9		6		4		5		2		5		1		2		9		11	
– 8.05	44– 48	7	19		12		4		6		6		6		7		6		10		7	
– 9.05	40– 43	2	15		5		3		8		5		11		3		12		2		8	
–10.05	36– 39	1	8		18				3		6		10		7		6		1		3	
–11.05	33– 35	1	1						2		2		1		4		1					
–12.05	30– 32		1		1																	
–13.05	27– 29				1																	
–14.05	24– 26																					
–15.05	22– 23																					
–16.05	20– 21																					
–17.05	18– 19																					
Total		441	58	0	62	15	12	38	25	2	24	7	39	17	23	17	29	9	28	17	44	7

TABLE 21 (continued)

Distribution of frequencies of factorial values. Low and high selection. Males. Direct lines only.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																				
		P	33		34		35		36		37		38		39		40		41		42	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye									1													
+14.95	439–484																					
+13.95	397–438																					
+12.95	359–396																					1
+11.95	325–358																					
+10.95	294–324	1		1		2		1														
+ 9.95	266–293			1																		
+ 8.95	241–265	3		1				2		3		1				1				1		
+ 7.95	218–240	6		3						6				1		2		1		5		1
+ 6.95	197–217	15		5		3		2		7		1		3		1		10		6		1
+ 5.95	178–196	16		4		2		1		5		3		2		3		8		6		5
+ 4 95	161–177	26		3		1		1		4				0		2		8		5		1
+ 3.95	146–160	19		7		1		1		4				2		9		2		5		3
+ 2.95	132–145	34		2		1				2						4		6				4
+ 1.95	119–131	35		1										1		1						1
+ 0.95	108–118	33				2								1								
− 0.05	98–107	**50**																				
− 1.05	89– 97	55																				
− 2.05	81– 88	37																				
− 3.05	73– 80	29																				
− 4.05	66– 72	23													1							
− 5.05	60– 65	22	1				2				9		3		2							
− 6.05	54– 59	18	1				3		2		9		3		7						6	
− 7.05	49– 53	8	4		3		4		6		10		3		9				3		4	
− 8.05	44– 48	7	11		2		3		10		5		5		9		1		5		9	
− 9.05	40– 43	2	9		8		5		17		5		14		12		3		9		5	
−10.05	36– 39	1	6		7		2		12				2		9		2		10			
−11.05	33– 35	1	2		1				1										3			
−12.05	30– 32																		1			
−13.05	27– 29		1																			
−14.05	24– 26																					
−15.05	22– 23																					
−16.05	20– 21																					
−17.05	18– 19																					
Total		441	35	28	21	12	19	8	49	31	38	5	30	10	49	23	6	35	31	28	24	17

Table 22

Distribution of frequencies of factorial values. Low and high selection. Females. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																				
		P	1		2		3		4		5		6		7		8		9		10	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1										1		1								
+20.07	397–438																					
+19.07	359–396																					
+18.07	325–358																					
+17.07	294–324																					
+16.07	266–293	1																				
+15.07	241–265											1										
+14 07	218–240																					
+13 07	197–217			1								1										
+12.07	178–196									1		10		1						1		
+11.07	161–177					1				2		11								6		1
+10.07	146–160					2		1		11		21		4						11		4
+ 9 07	132–145	1		4		10		3		18		22		9		12		2		17		8
+ 8.07	119–131			6	1	17		16		29		20		12		8		2		17		23
+ 7.07	108–118	7		7		27		23		32		31		17		10		14		15		20
+ 6.07	98–107	11		16		31		26		28		26		23		14		11		24		16
+ 5.07	89– 97	27		16	7	33		22		19		9		12		17		13		16		9
+ 4.07	81– 88	25		20	16	14		14	1	6		3		4		8	1	9	1	17	1	1
+ 3.07	73– 80	29		15	11	10		4	2	1		2				10	5	12	2	4	7	
+ 2.07	66– 72	51	3	17	25	4		5	3	1	1		2			3	11	3	7	3	10	
+ 1.07	60– 65	55	7	20	30	11	6	2	3			1	5			4	10		10	2	19	
+ 0.07	54– 59	54	15	36	34	1	10	1	4		1		8		8	3	28		29		24	
– 0.93	49– 53	58	21	18	23	1	28	1	3		1		4		10	1	24		33		24	
– 1.93	44– 48	54	14	22	22	1	20		3		4		8		6	1	11		27		28	
– 2.93	40– 43	47	13	5	4		17		5		5		14		8		4		9		7	
– 3.93	36– 39	40	30	3	3	1	15		3		1		4		8				1		2	
– 4.93	33– 35	15	5	1	1		4		2				2		5		2					
– 5.93	30– 32	9	3		1				1						2							
– 6.93	27– 29	3																				
– 7.93	24– 26	1															1					
– 8.93	22– 23																					
– 9.93	20– 21	1																				
–10.93	18– 19																					
Totals		490	111	207	178	164	100	118	30	148	13	159	47	83	47	91	97	56	119	133	122	82

Table 22 (continued)

Distribution of frequencies of factorial values. Low and high selection. Females. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																				
		P	11		12		13		14		15		16		17		18		19		20	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1			1																	
+20.07	397–438																					
+19.07	359–396																					
+18.07	325–358																					
+17.07	294–324																					
+16.07	266–293	1																				
+15.07	241–265													1								
+14 07	218–240													2								
+13.07	197–217													2								
+12.07	178–196													6		1						1
+11.07	161–177			3										3								2
+10.07	146–160			2		3				3		1		7		2						3
+ 9 07	132–145	1		9		4		5		17		2		13		4		2		1		14
+ 8.07	119–131			18		13		20		**21**		16		15		15		4				22
+ 7 07	108–118	7		**28**		**14**		**22**		20		**10**		**16**		**12**		3		4		**33**
+ 6 07	98–107	11		50		11		29		5		21		31		11		**5**		**3**		33
+ 5.07	89– 97	27		15		6		5		5		8		30		6		3		4		22
+ 4 07	81– 88	25		7	1	1		3						15		4		2				12
+ 3.07	73– 80	29	2	3				1				1		2		3		2				2
+ 2 07	66– 72	51	8				1					1		2	1	1		1				2
+ 1.07	60– 65	55	5		3		3								5		1					
+ 0 07	54– 59	**54**	17		16		12		3		1		5		19	1	3		3		1	1
– 0 93	49– 53	58	13		21		10		9		5		8		21		8		14		8	
– 1 93	44– 48	54	18		**24**		19		22		16		16		**23**		20		45		19	
– 2.93	40– 43	47	4		19		43		**26**		**36**		14		17		**21**		**22**		**11**	
– 3 93	36– 39	40	2		8		13		29		34		18		7		20		30		12	
– 4.93	33– 35	15	3		4		5		7		10		6		4		3		4		8	
– 5.93	30– 32	9			1		3				2		4		1		3		4		1	
– 6.93	27– 29	3									1						1					
– 7.93	24– 26	1			1												1					
– 8.93	22– 23																					
– 9.93	20– 21	1																				
–10.93	18– 19																					
Totals		490	72	135	99	52	109	85	96	71	105	60	71	145	98	60	81	22	122	12	60	147

TABLE 22 (continued)

Distribution of frequencies of factorial values. Low and high selection. Females. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																					
		P	21		22		23		24		25		26		27		28		29		30		31	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1												1										
+20.07	397–438																							1
+19.07	359–396																							
+18.07	325–358																							
+17.07	294–324																	1						
+16.07	266–293	1		1														3		1				
+15.07	241–265											1				1		5		4		2		
+14.07	218–240					1								1		3		2		5		4		3
+13.07	197–217			1		1				3		3		9		1		9		6		3		7
+12.07	178–196			2		1				1		7		11		4		8		8		7		9
+11.07	161–177			4		2		2		4		7		7		3		11		11		13		15
+10.07	146–160			3		9		2		9		8		6		1		5		8		9		9
+ 9.07	132–145	1		21		12		11		20		16		17		7		7		2		4		10
+ 8.07	119–131			26		13		21		22		19		8		1		1		2		4		12
+ 7.07	108–118	7		22		15		12		12		20		12		3		3				3		5
+ 6.07	98–107	11		19		6		11		16		17		1		1				1				4
+ 5.07	89– 97	27		10		13		13		8		12				3		1						2
+ 4.07	81– 88	25		6		5		4		6		3		1		2		1						1
+ 3.07	73– 80	29						1		1														1
+ 2.07	66– 72	51				5				1			1											1
+ 1.07	60– 65	55	1			1		1	1		3				1									
+ 0 07	54– 59	54	1			1			2		4	1											3	
– 0.93	49– 53	58	4		4		2		9		10		7		5		5		1				9	
– 1.93	44– 48	54	8		9		20		13		30		17		21		5		13		4		27	
– 2.93	40– 43	47	23		13		24		11		24		20		22		16		15		11		23	
– 3.93	36– 39	40	31		5	1	17		34		17		12		28		19		16		13		14	
– 4.93	33– 35	15	6				13		21		6		1		12		5		3		5		8	
– 5.93	30– 32	9	4				5		13		1				1		4		3				4	
– 6 93	27– 29	3					1		11						2									
– 7.93	24– 26	1							8															
– 8.93	22– 23								4															
– 9.93	20– 21	1							2															
–10.93	18– 19																							
Totals		490	78	115	31	86	82	79	129	103	95	114	58	74	92	30	54	57	51	48	33	49	88	80

TABLE 22 (continued)

Distribution of frequencies of factorial values. Low and high selection. Females. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																						
		P	32		33		34		35		36		37		38		39		40		41		42	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Heterozygotes		1	1									1												
+20.07	397–438																							
+19.07	359–396																							
+18.07	325–358																							
+17.07	294–324																							
+16.07	266–293	1																						
+15.07	241–265					1						1												
+14.07	218–240			1		1		1																
+13.07	197–217			2		4		2		1						1								
+12 07	178–196			15		8		2		5		2		3		1		1		1		1		
+11.07	161–177			12		11		9		9		11		15		4		8		8		7		
+10 07	146–160			7		20		13		7		8		10		9		10		12		7		
+ 9 07	132–145	1		14		14		17		14		11		10		9		9		23		3		4
+ 8.07	119–131			7		9		25		6		13		2		2		4		12				2
+ 7.07	108–118	7		5		13		12		7		11		2		2		2		9				2
+ 6 07	98–107	11		5		7		5		8		6				2		4		4				1
+ 5.07	89– 97	27		1		4		8		3		7												
+ 4.07	81– 88	25				1		4		4														
+ 3.07	73– 80	29	1	1						1		1												
+ 2.07	66– 72	51						1		1		1												
+ 1 07	60– 65	55	2	1											1									
+ 0.07	54– 59	54	4						3		1		2		1				1		1			
– 0 93	49– 53	58	12								3		6		1		3		8				2	
– 1.93	44– 48	54	25		2		1		7		8		17		10		12		19		2		11	
– 2.93	40– 43	47	31		9		12		17		21		17		15		10		33		11		8	
– 3.93	36– 39	40	24		21		11		20		31		14		4		24		21		9		10	
– 4.93	33– 35	15	9		11		4		8		23		5		1		10		2		5		1	
– 5.93	30– 32	9			5		5		5		1		2		1		6		1		1			
– 6.93	27– 29	3			3				2		2						4				1			
– 7.93	24– 26	1			1												1							
– 8.93	22– 23																				1			
– 9.93	20– 21	1																						
–10.93	18– 19										1													
Totals		490	109	71	52	93	33	99	62	66	91	73	63	42	34	30	70	38	85	69	31	18	32	9

TABLE 23

Distribution of frequencies of factorial values. Low and high selections. Males. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																				
		P	1		2		3		4		5		6		7		8		9		10	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye																						
+14.95	439–484											1										
+13.95	397–438											3										
+12.95	359–396											2										
+11.95	325–358											2								1		
+10 95	294–324	1																		2		3
+ 9.95	266–293											3								2		1
+ 8.95	241–265	3								1		11		1						7		2
+ 7.95	218–240	6		1		2						13		2		1		1		5		6
+ 6 95	197–217	15		4		5		1		4		19								6		11
+ 5.95	178–196	16		3		4		1		7		20		2		11		4		13		14
+ 4.95	161–177	26		6		5		3		20		26		16		15		9		19		20
+ 3 95	146–160	19		6		9		15		22		28		17		18		6		17		18
+ 2 95	132–145	34		13	2	24		17		25		27		24		15		4		16		11
+ 1 95	119–131	35	1	15	3	17		21		34		7		21		10		8		15		5
+ 0.95	108–118	33	2	19	6	19		25		15		6		6		6		9		8	1	7
− 0.05	98–107	50	3	23	8	7		19		16				4		11		5	1	13	4	3
− 1.05	89– 97	55	10	13	15	3	1	10		1		1			1	7		1	1	3	7	
− 2 05	81– 88	37	6	24	26	13	1	7			2		1		3	1	2	2		1	12	
− 3 05	73– 80	29	13	14	23	7	4	2	3	2	1		8		1	1	5		4		19	
− 4 05	66– 72	23	25	28	18	8	7	2	7		2		4		6		26		13		12	
− 5.05	60– 65	22	17	15	21	3	12		5		1		8		4		16		20		13	
− 6.05	54– 59	18	17	11	19		21		11		3		4		11		15		35		21	
− 7.05	49– 53	8	8	6	7	1	14	1	7		3		6		2		11		21		16	
− 8.05	44– 48	7	10	8	7		11	1	4				2		4		11		18		5	
− 9 05	40– 43	2	7		2		6	1	4		2		3				1		2		1	
−10.05	36– 39	1	3				3				1		2				1					
−11.05	33– 35	1	2				1															
−12.05	30– 32																					
−13.05	27– 29																					
−14.05	24– 26																					
−15 05	22– 23																		1			
−16.05	20– 21																					
−17.05	18– 19				1																	
−18.05	16– 17																					
Totals		441	124	209	158	127	81	126	41	147	15	169	39	93	32	96	88	49	116	128	111	101

TABLE 23 (continued)

Distribution of frequencies of factorial values. Low and high selection. Males. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																				
		P	11		12		13		14		15		16		17		18		19		20	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye																						
+14 95	439–484																					
+13.95	397–438																					
+12 95	359–396					1																
+11.95	325–358					1																
+10 95	294–324	1																1				
+ 9.95	266–293					1				1				2								
+ 8.95	241–265	3				2								2				1				
+ 7.95	218–240	6		8		6		1		2				6		3		2				
+ 6 95	197–217	15		10		7		8		8		1		13		2		3		1		5
+ 5.95	178–196	16		14		**11**		7		13		5		14		7						8
+ 4 95	161–177	26		28		3		14		**20**		7		16		7		1				15
+ 3.95	146–160	19		**35**		9		**21**		13		13		**25**		**13**		**2**		4		30
+ 2.95	132–145	34		21		4		19		5		**13**		24		20		5		**5**		**18**
+ 1 95	119–131	35		10		2		10		2		12		28		4		3		4		21
+ 0 95	108–118	33		15		2		6		1	1	8		18		6		1		1		9
– 0 05	98–107	**50**	1	11				1				2		8	2	2		1		2		8
– 1.05	89– 97	55	4	1								2		2	1		1			1		4
– 2 05	81– 88	37	6	1	1		1								1		1	1	2			
– 3 05	73– 80	29	9		3		7				3				4		1		2			
– 4 05	66– 72	23	13		9		8				2		1		2				2		1	
– 5 05	60– 65	22	**6**		19		21		3		7		4		4		3		9		1	
– 6 05	54– 59	18	4		21		19		5		13		10		23		5		16		3	
– 7.05	49– 53	8	4		**24**		**17**		14		19		12		**21**		15		18		10	
– 8 05	44– 48	7	6		18		22		**30**		**40**		18		20		**33**		**29**		15	
– 9 05	40– 43	2	7		13		17		25		28		**22**		16		17		25		**19**	
–10 05	36– 39	1					4		24		20		21		7		10		30		18	
–11 05	33– 35	1							3		6		9		3				4		2	
–12 05	30– 32								1				5				1					
–13.05	27– 29				1																	
–14.05	24– 26																					
–15.05	22– 23																					
–16.05	20– 21																					
–17.05	18– 19																					
–18 05	16– 17																					
Totals		441	60	154	109	49	116	87	105	65	139	63	102	158	104	64	87	21	137	18	69	118

TABLE 23 (continued)

Distribution of frequencies of factorial values. Low and high selection. Males. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																						
		P	21		22		23		24		25		26		27		28		29		30		31	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye																								
+14.95	439-484																							
+13.95	397-438																							1
+12.95	359-396																							
+11.95	325-358			1																1				
+10.95	294-324	1		1				1																
+ 9.95	266-293									1				2				1		3		2		1
+ 8.95	241-265	3				2		1		2		1		4		2		3		4				2
+ 7.95	218-240	6		7		4		2		5		2		3		3		3		4		5		5
+ 6.95	197-217	15		15		5		13		8		14		9		3		2		6		6		6
+ 5.95	178-196	16		10		8		16		16		11		5		4		3		9		8		12
+ 4.95	161-177	26		16		14		17		19		25		7		2		4		3		4		8
+ 3.95	146-160	19		12		15		18		14		15		8		1		2		2		6		9
+ 2.95	132-145	34		11		11		12		12		15		5		1		1		2		1		7
+ 1.95	119-131	35		3		2		17		7		4		2		3								3
+ 0 95	108-118	33		2		2		7		3		5		2		1								
− 0.05	98-107	50		1		2		4		2		1												
− 1.05	89- 97	55								1	1													
− 2.05	81- 88	37						1			1		1											
− 3.05	73- 80	29							3		1				1		1							
− 4.05	66- 72	23	1		1		2		3		3		2		1				2				2	
− 5.05	60- 65	22	3		1		5		8		5		4				2		1				2	
− 6 05	54- 59	18	5		5		7		9		17		10		10		4		4		2		11	
− 7.05	49- 53	8	5		13		18		13		24		11		5		7		7		3		24	
− 8.05	44- 48	7	32		16		29		29		29		14		18		8		16		11		23	
− 9 05	40- 43	2	28		11		23		22		26		9		19		11		11		17		16	
−10.05	36- 39	1	45		2		13		29		17		3		20		10		12		8		3	
−11.05	33- 35	1	12				2		3		2		2		5		1		4		1		1	
−12.05	30- 32		4				1		3						1								1	
−13.05	27- 29		1						1															
−14.05	24- 26																							
−15.05	22- 23																							
−16 05	20- 21																							
−17.05	18- 19																							
−18.05	16- 17								1															
Totals		441	136	79	49	65	100	109	124	90	126	93	56	47	80	20	44	19	57	34	42	32	83	54

TABLE 23 (continued)

Distribution of frequencies of factorial values. Low and high selection. Males. All matings.

CLASS VALUES IN FACTORIAL UNITS	CLASS RANGES IN FACET NUMBERS	FREQUENCIES																						
		P	32		33		34		35		36		37		38		39		40		41		42	
			L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
Full eye											1													
+14.95	439–484																							
+13.95	397–438																							
+12.95	359–396							1																
+11.95	325–358																			1				1
+10.95	294–324	1				1		2		1														
+ 9.95	266–293					1		1																
+ 8.95	241–265	3		2		1		1		2		3		3				2		2		1		
+ 7.95	218–240	6		1		3		2				7		8		3		4		5		5		1
+ 6.95	197–217	15		3		6		11		8		13		8		8		10		17		8		1
+ 5.95	178–196	16		2		10		14		8		10		10		5		7		20		8		5
+ 4.95	161–177	26		9		8		16		14		13		7		3		10		21		5		1
+ 3.95	146–160	19		3		13		14		10		13		1		5		13		14		5		3
+ 2.95	132–145	34		6		7		8		7		9		2		3		8		20				4
+ 1.95	119–131	35				3		5		5		8				1		1		2				1
+ 0.95	108–118	33				2		9				2				1				2				
– 0.05	98–107	50	1			1		2				2												
– 1.05	89– 97	55	1							1														
– 2 05	81– 88	37										1												
– 3 05	73– 80	29	1						1															
– 4.05	66– 72	23	2						2								1							
– 5.05	60– 65	22	1		1				4				13		3		2							
– 6.05	54– 59	18	14		1				7		3		12		3		8		7		2		6	
– 7 05	49– 53	8	17		4		4		13		6		15		3		10		3		5		4	
– 8.05	44– 48	7	23		18		4		17		19		17		5		20		34		7		9	
– 9.05	40– 43	2	42		11		15		17		30		15		14		23		24		14		5	
–10.05	36– 39	1	12		13		10		10		27		5		2		12		9		17			
–11.05	33– 35	1	2		4		1				7						1				4			
–12.05	30– 32		1						1												1			
–13.05	27– 29				1																			
–14.05	24– 26																							
–15.05	22– 23																							
–16.05	20– 21															1								
–17.05	18– 19																							
–18.05	16– 17																							
Totals		441	117	26	53	56	34	86	72	56	93	81	77	39	30	30	77	55	77	104	50	32	24	17

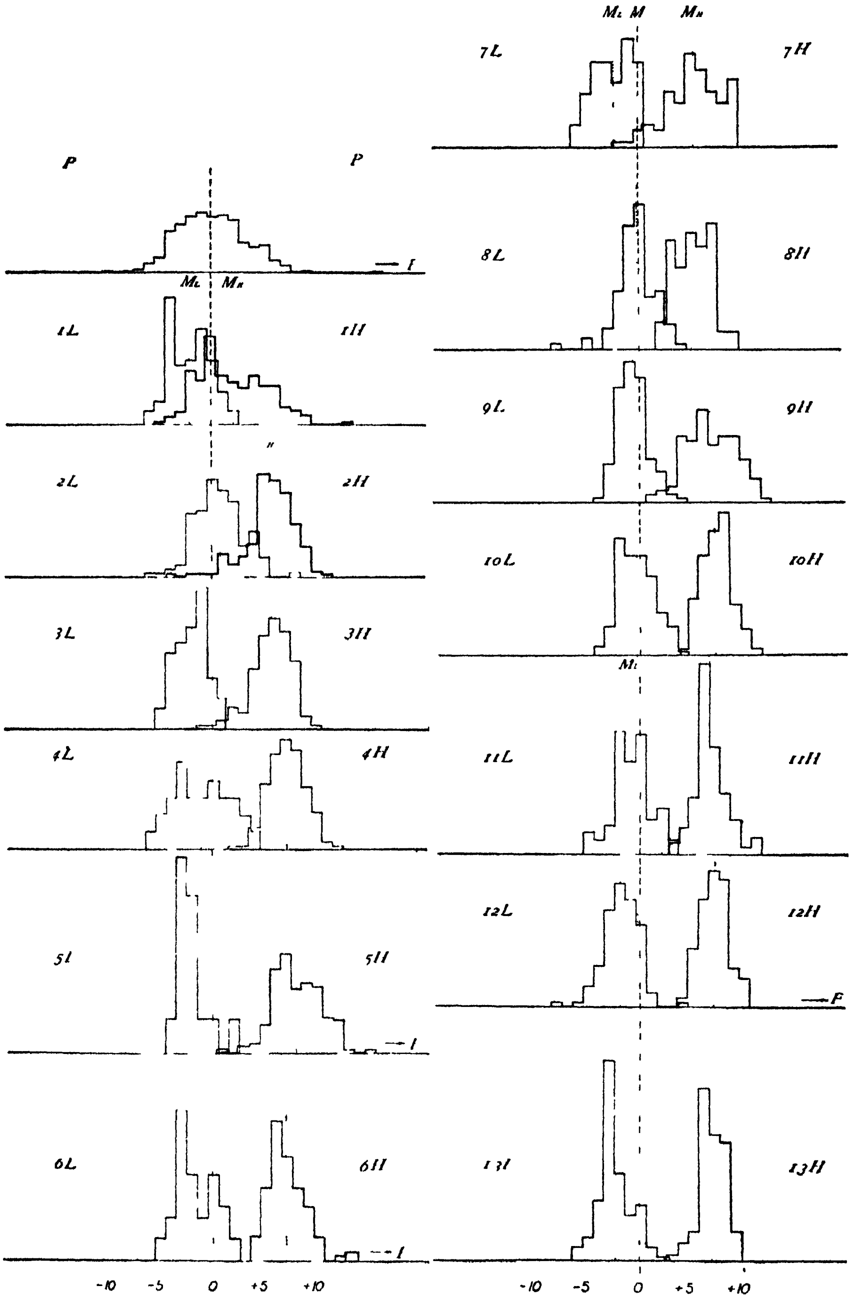
for low line (L) and high line (H) the numbers of offspring to be found within the limits of each class. The basis of reference is the mean value of the unselected population and the class corresponding to this value is inclosed in two horizontal lines. In each generation for both low and high lines the frequency of the class which includes the mean value for that generation is put in bold-faced type so that in a general way the effect of selection may be followed.

Because of the change in sexual dimorphism during the course of selection, a proper picture of the effect of selection can not be drawn from one sex alone. The high-line values of the females depart from the unselected population more widely than those of the low line, while the reverse is true of the males. Since all the offspring of each mating are included, it is possible by comparing the two tables to obtain the relative numbers of females and males. In the direct line where only a single brother or sister mating is involved, this ratio is of value in determining the presence of lethal mutant factors. A number of these are indicated by the data. Some are of the usual type with twice as many females as males. Others are of the type recently discovered by THOMPSON (1921) with twice as many males as females.

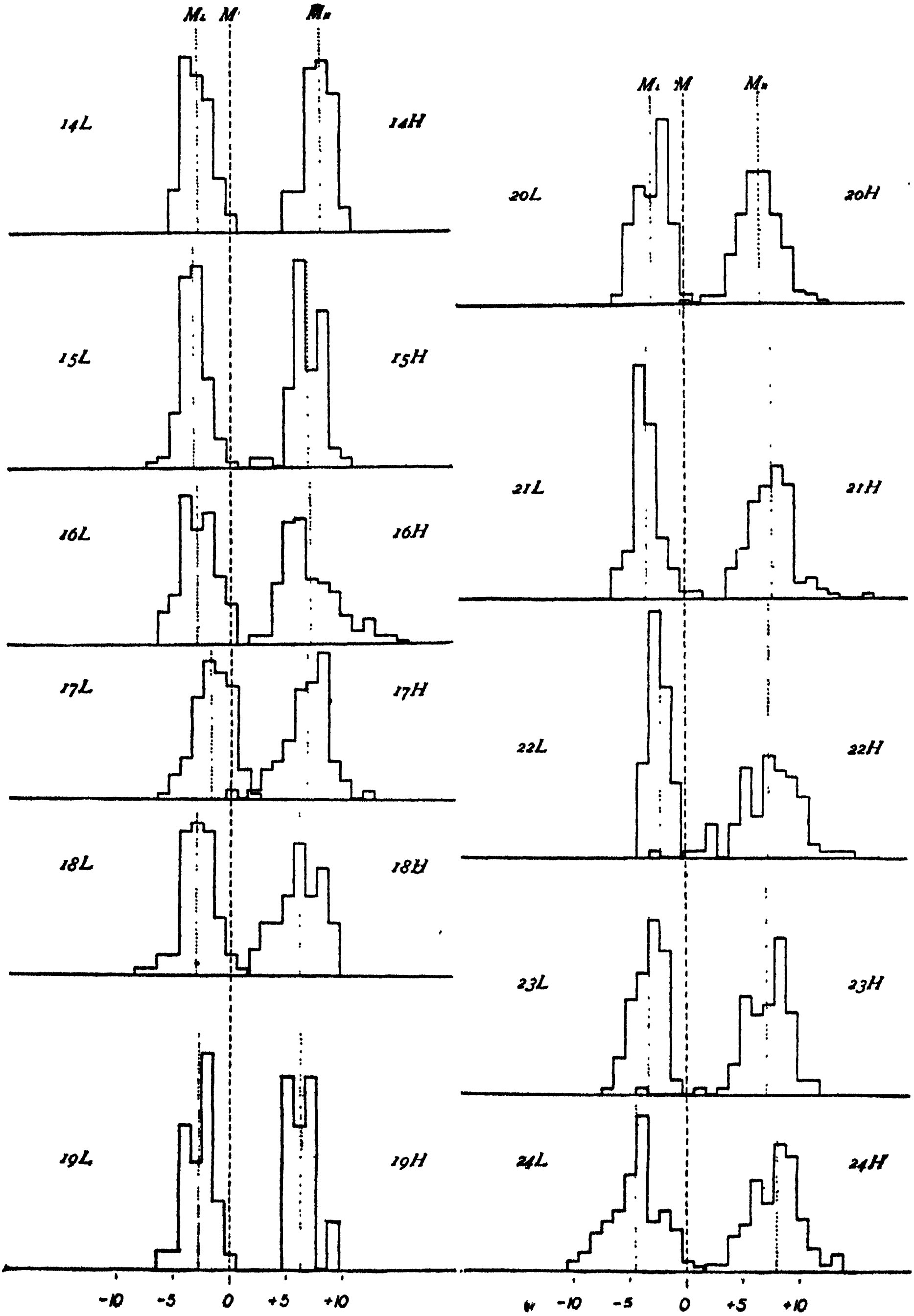
Distribution of frequencies for "all matings"

When all matings are included there is the advantage of the large numbers of individuals and the disadvantage of less uniformity. The data are given in table 22 for the females and table 23 for the males. The arrangement is the same as for the direct-line generations. The divergence of the high and low lines is clearly indicated, as is the general failure of the selection generations to extend their range beyond that of the original population.

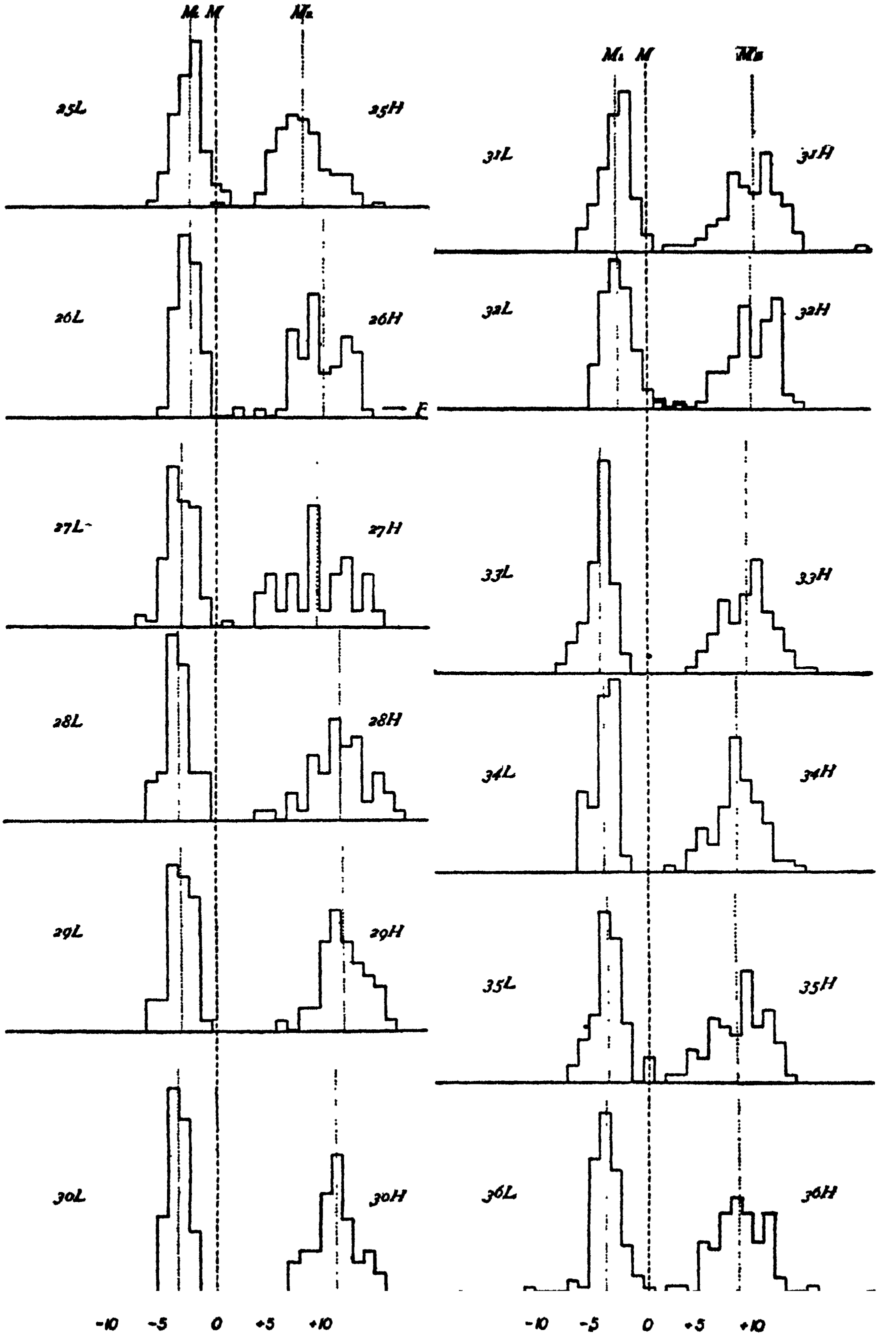
The data are given in graphic form in figures 12 to 54 for the females and figures 55 to 97 for the males. In each figure the horizontal scale represents the classes in terms of departure from the mean of the unselected population (M), the zero of the scale, which is indicated by the central dotted line. The mean of the low selection (M_L) is shown by the dotted line at the left, and the mean of the high selection (M_H) by the dotted line at the right. The distance between the two is the effectiveness of selection up to the given generation in terms of 10-percent factorial units. The vertical distances in the graphs represent the percentages of the individuals to be found in each class.



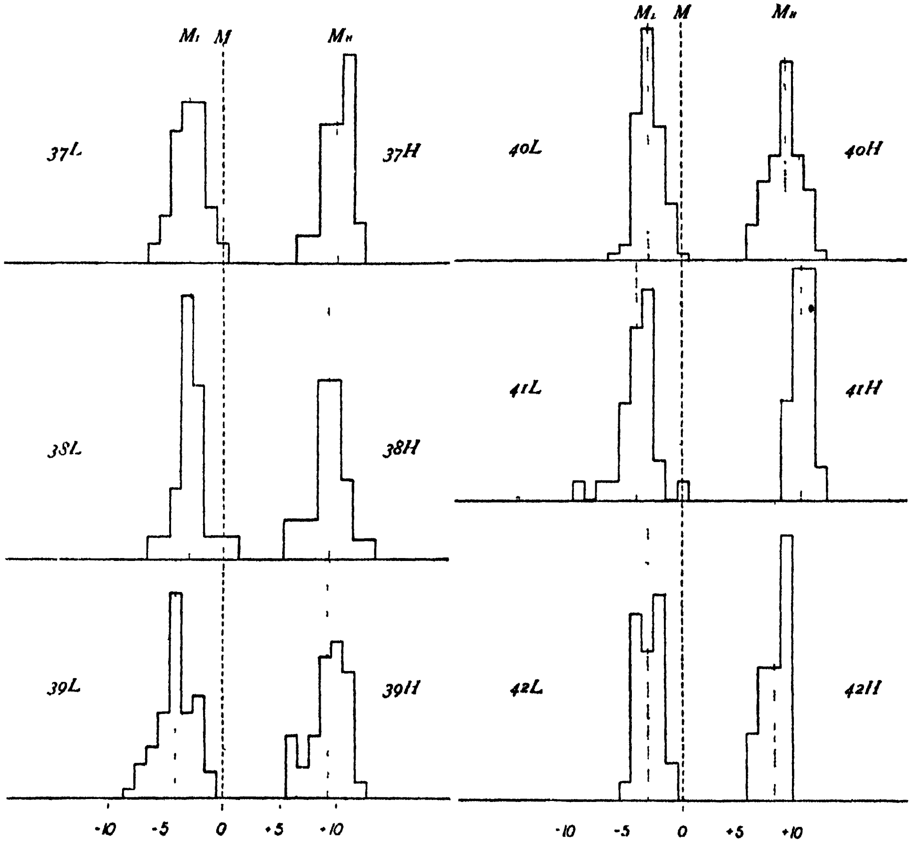
FIGURES 12-25.—Frequency distributions of factorial values in females of the parental unselected population and of selection generations 1 to 13. M, the central dotted line, is the mean of the parental unselected population. Of the other two dotted lines, the left-hand one is the mean of the low-selection line and the right-hand one the mean of the high-selection line. The scale of factorial values is given at the bottom of each row of figures and has the mean of the unselected population as its zero and departures are represented in factorial units, each unit representing a factor capable of producing a ten-percent change in facet value.



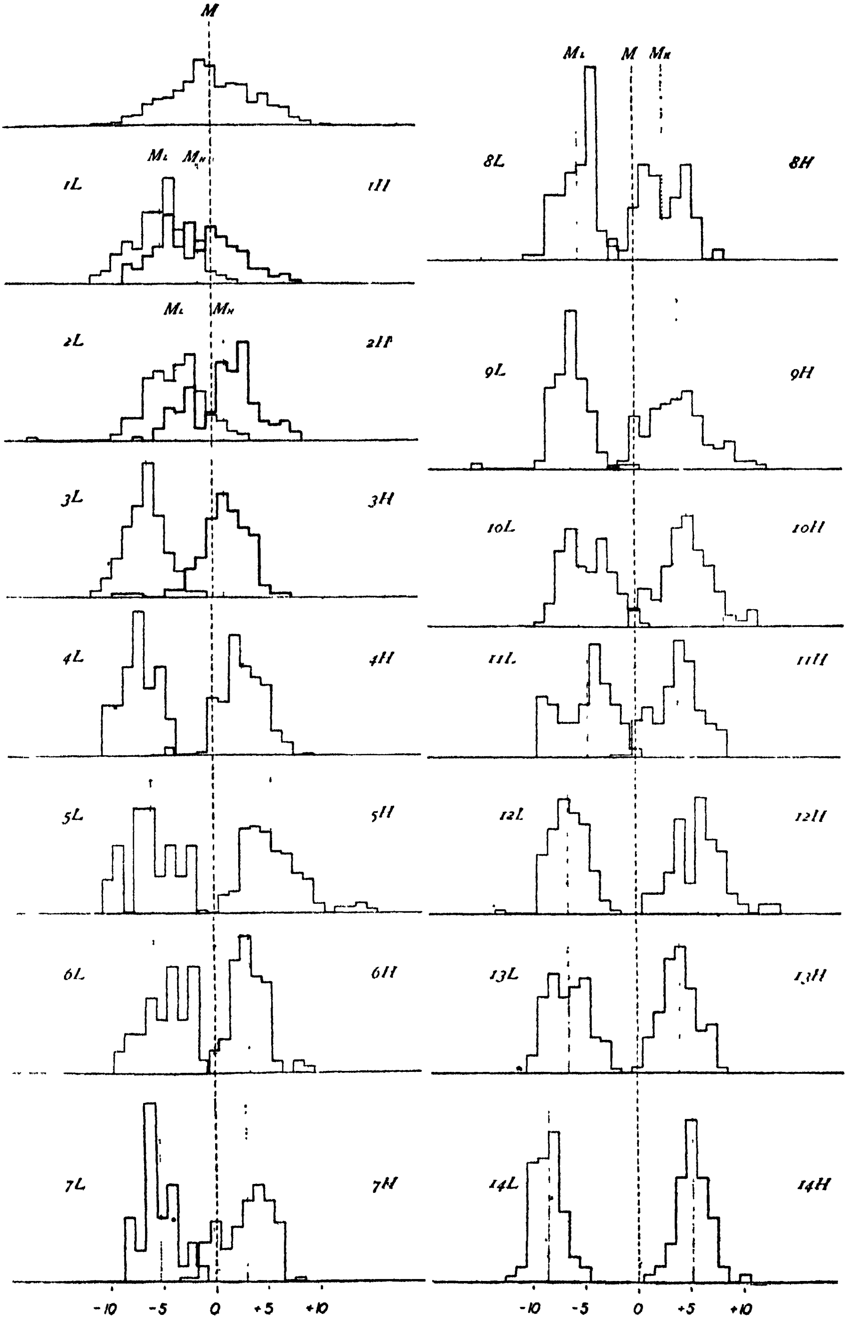
Figures 26–36.—Frequency distributions of factorial values in females of selection generations 14 to 24.



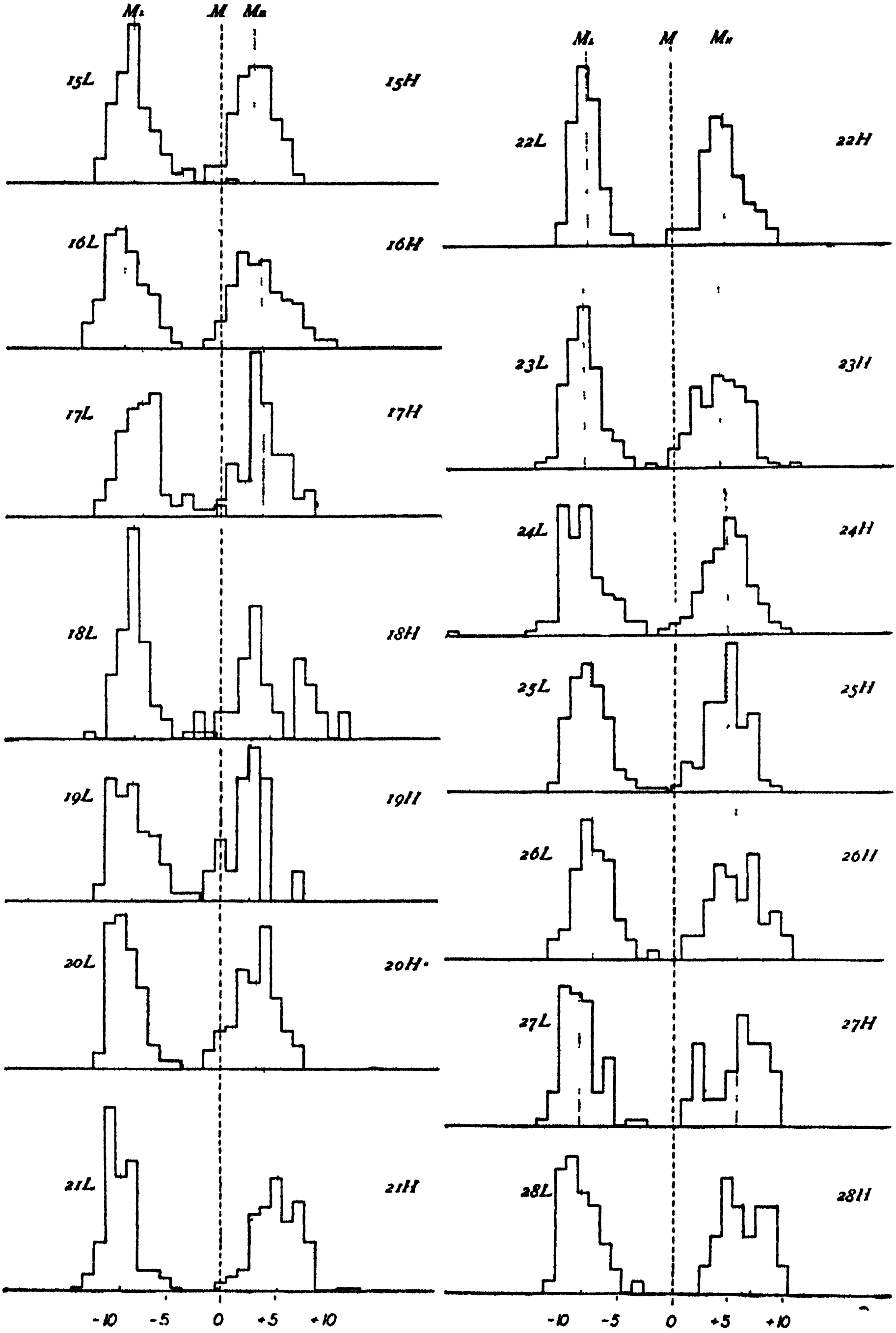
FIGURES 37–48.—Frequency distributions of factorial values in females of selection generations 25 to 36.



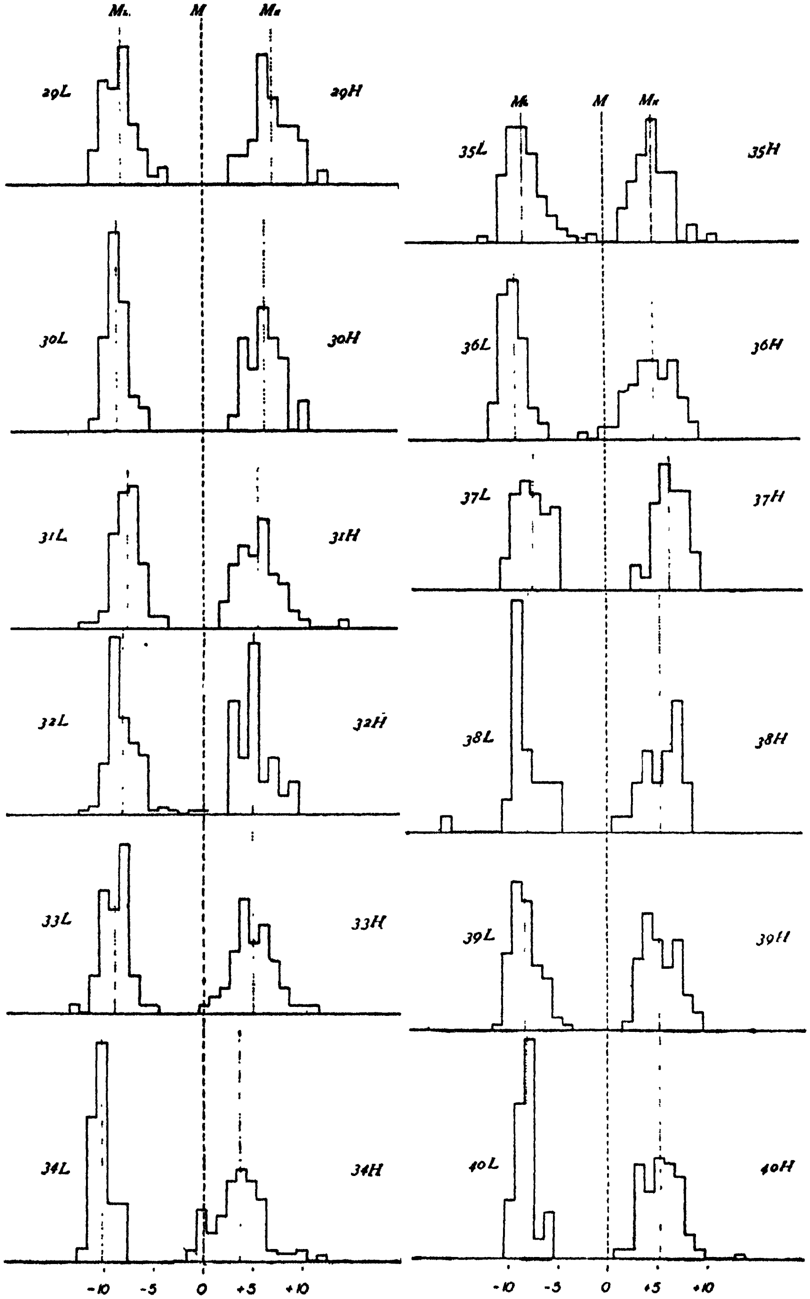
FIGURES 49-54.—Frequency distribution of factorial values in females of selection generations 37 to 42.



FIGURES 55–69.—Frequency distributions of factorial values in males of the parental unselected population and of selection generations 1 to 14.



Figures 70–83.—Frequency distributions of factorial values in males of selection generations 15 to 28.



FIGURES 84–95.—Frequency distributions of factorial values in males of selection generations 29 to 40.

In considering these graphs, it is again important to bear in mind the decrease in sexual dimorphism during the course of selection, which makes it appear that the high selection is more effective than the low in the females and the low more effective than the high in the males. The graphs show very clearly that there is no pronounced increase in total range as a result of selection, but that there is practically a complete separation in range of the high and low lines after the first few generations. Since they are constructed on the factorial basis, they are strictly comparable throughout. They also bring out in a striking manner the decrease in variability during the early generations of selection.

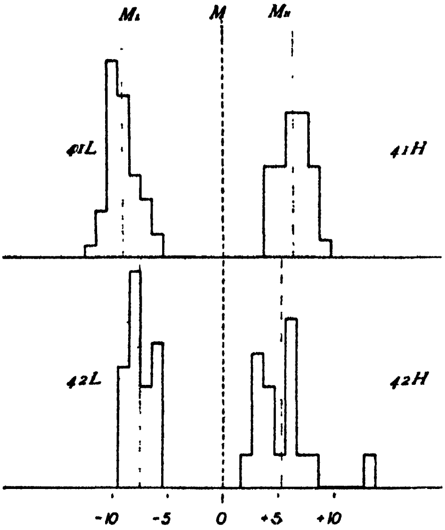


FIGURE 96–97.—Frequency distributions of factorial values in males of selection generations 41 and 42.

RESULTS

Range

That the effect of selection is due to the elimination of the high germinal groups in the low line without essential modification of the existing low groups and to the elimination of low germinal groups in the high line without essential modification of existing high groups is well illustrated by the ranges as given in tables 12 to 15 and figures 98, 100, 102 and 104 for the direct line, and tables 16 to 19 and figures 99, 101, 103 and 105 for "all matings." In the figures the vertical scale represents factorial values, the zero of the scale being the mean of the unselected population. The values of the highest and lowest individuals in the unselected population are represented on this scale under P. The continuous horizontal line (HV) extending from the upper value makes it available for reference throughout the selection generations, and one can determine directly the modification of the original upper extreme in each generation. The lower continuous horizontal line (LV) serves in the same way for the lower extreme. The upper zigzag line follows the values of the highest individual in each generation, HL in the low-selection line and HH in the high line. The lower zigzag line follows the values of the lowest individual, LL in the low-selection line and LH in the high line.

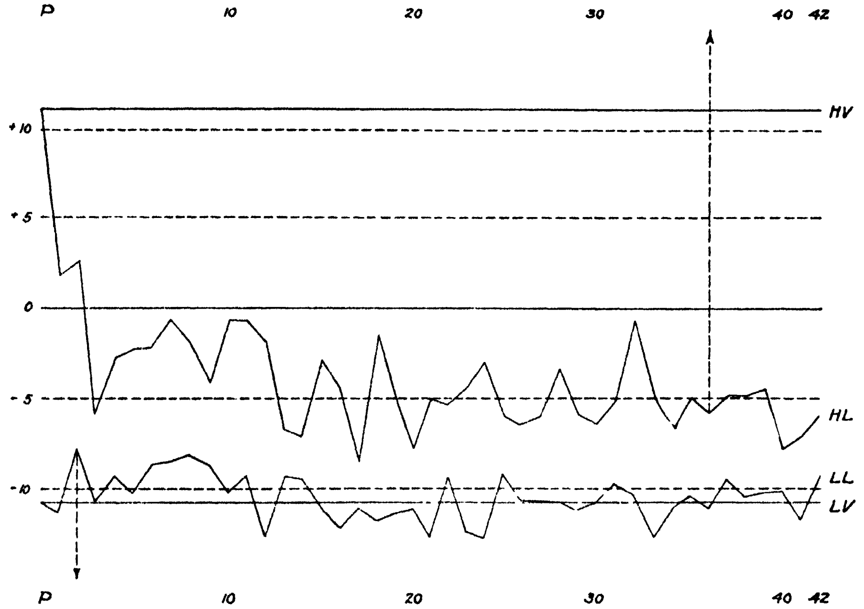


Figure 98.—Upper and lower limits of range in males of the direct low line. The vertical scale is in factorial units, the zero of the scale being the mean of the unselected population. The values of the highest and lowest individuals in the unselected population are represented on this scale under P. The continuous horizontal line (HV) extending from the upper value makes it available for reference throughout the selection generations, and one can determine directly the modification of the original upper extreme in each generation. The lower continuous horizontal line (LV) serves in the same way for the lower extreme. The upper zigzag line (HL) follows the values of the highest individual in each generation. The lower zigzag line (LL) follows the values of the lowest individual in the same way.

It is evident at a glance that the values of the lowest individuals of the unselected population have not been made still lower as a result of selection in the low line, nor have the values of the highest individuals with an exception to be mentioned later, been made still higher in the high line. The result of selection is accomplished by the elimination of the high individuals in the low line and of the low individuals in the high line.

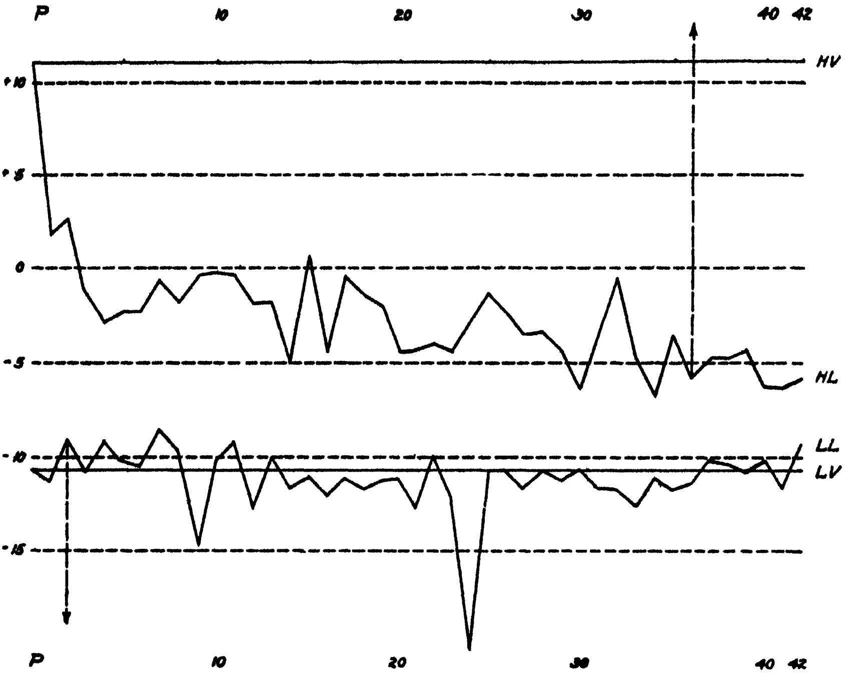


FIGURE 99.—Upper and lower limits of range in males of the low line including all matings.

In a study of the ranges it is necessary to bear in mind certain facts. First, the upward mutations to full eye and the downward ones to ultra-bar are purposely omitted in the figures except for the upwardly and downwardly directed dotted arrows which indicate their location. If they were included in the present discussion, obviously the effect of selection would be to bring bar very quickly up to full eye on the one hand and down to ultra-bar on the other. The transformation to full eye would have been accomplished in the first generation of upward selection and that to ultra-bar in the third generation of downward selection because a mutation to full appeared in the parental generation and one to ultra-bar in the second low selection. Some very low individuals which could not be tested because of early death or sterility may be

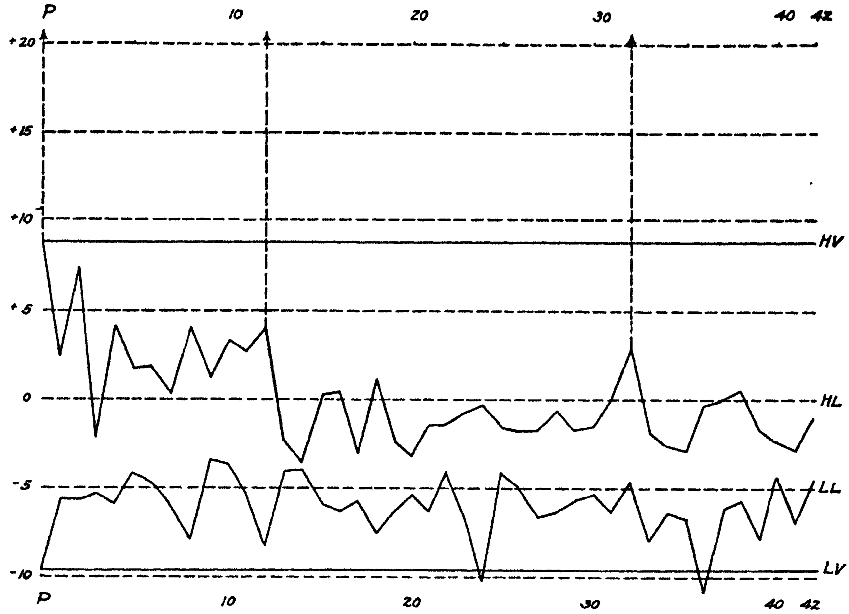


FIGURE 100 —Upper and lower limits of range in females of the direct low line.

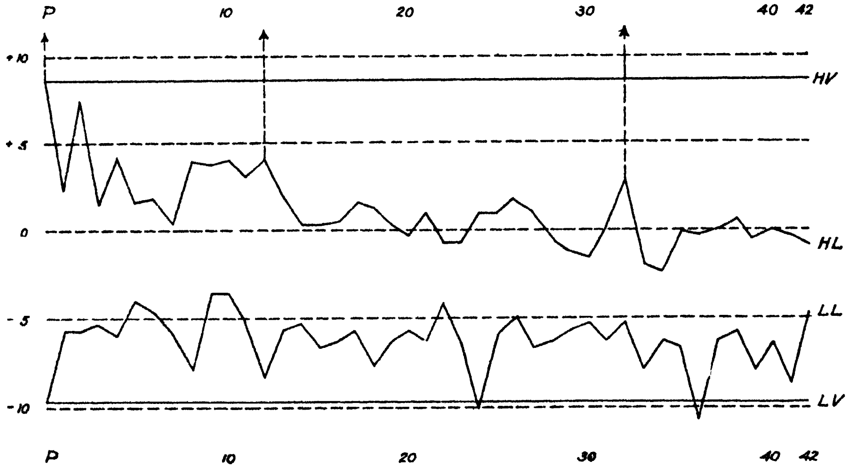


FIGURE 101.—Upper and lower limits of range in females of the low line including all matings.

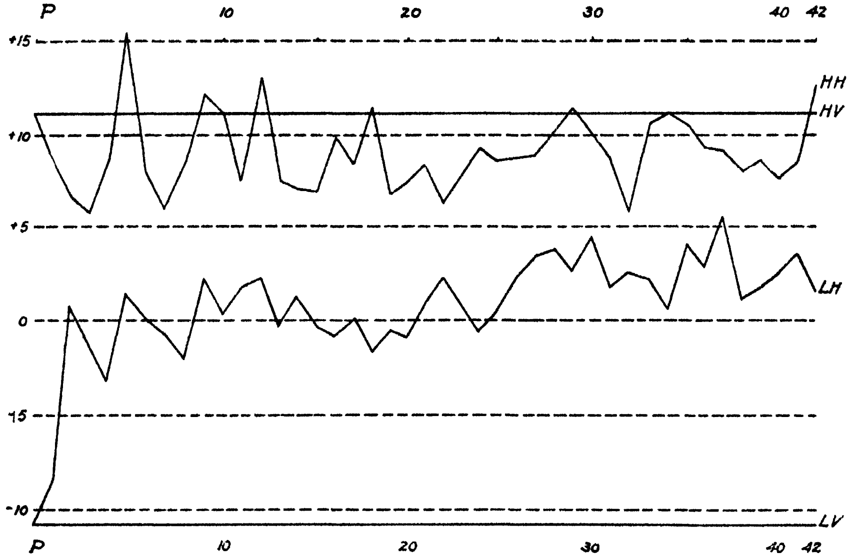


FIGURE 102.—Upper and lower limits of range in males of the direct high line. Arrangement as in figure 98 except that the values of the upper extremes are designated by HH and of the lower extremes by LH.

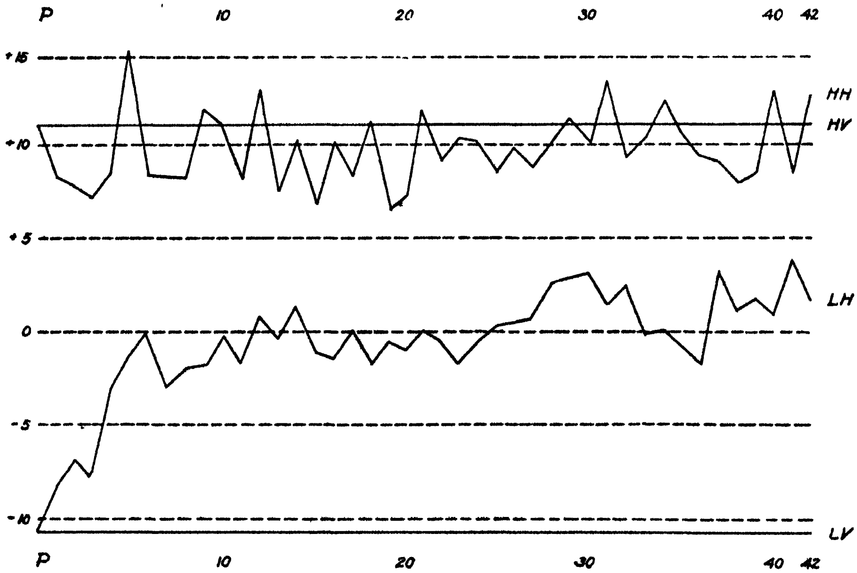


FIGURE 103.—Upper and lower limits of range in males of the high line including all matings.

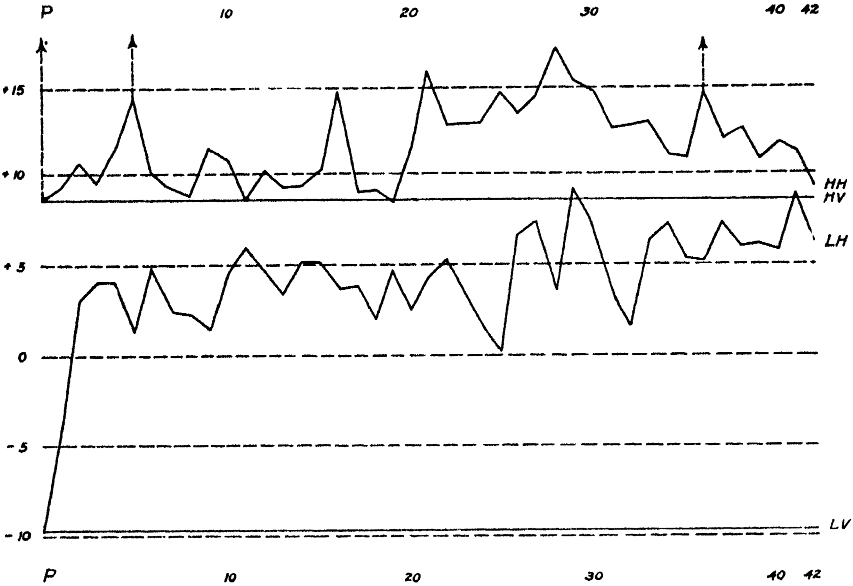


FIGURE 104.—Upper and lower limits of range in females of the direct high line.

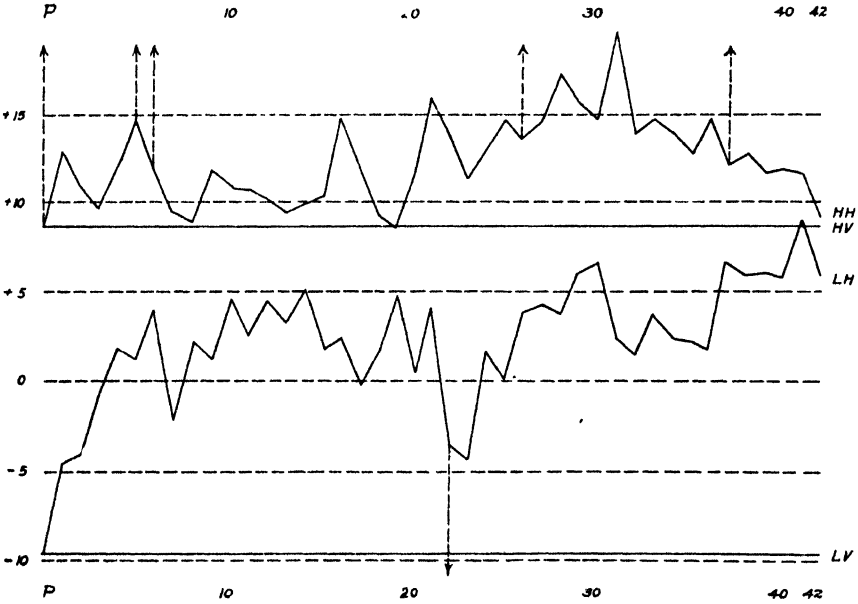


FIGURE 105.—Upper and lower limits of range in females of the high line including all matings.

ultra-bars, but in the absence of complete demonstration of their character they are included with the regular series of bars.

Second, the number of individuals in the parental generation is greater than in any one of the selection generations. Therefore the range of values should be less in individual generations of the latter than in the former. A part of the apparent restriction in range may be due to this cause. It is the probable explanation of the fact that the highest males in the high line are so frequently slightly lower than the highest male in the unselected population.

Third, as shown elsewhere (ZELENY 1921 b) the decrease in heterozygosis following selection with inbreeding increases the facet number of the females. This is not to be confused with the ordinary selection effect as the males are not involved. One of its results is to raise the level of all females and this is reflected in the figures of range by an exaggeration of the sorting effect in the females of the high line and an under-valuation of it in the low line. Likewise, it produces a false appearance of elimination of low extreme females in the low line and of extension of range upward in the high line.

Turning to a more detailed examination of the figures, it may be well to take up the males first because they do not show the change with decreased heterozygosis just described for the females. In the low direct line (figure 98) it is clear that neglecting the single mutation to ultra-bar in the second generation there has been no extension of the range downward. The lowest individuals (LL) in the selection generations follow very closely the lowest one in the unselected population which had a value of −10.72. The highest individuals (HL), however, drop very rapidly in value during the 1st and 3rd generations from +11.11 to −5.63, after which they fluctuate around the value of −8.00 until the 12th generation following which there is a further drop of about three units to a new level This last lowering of the high extreme is to be considered as a significant one, especially as it is reflected in a change in the mean and standard deviation. It is probable, therefore, that at this time selection has taken hold of one of the smaller germinal differences which had up to this time been masked by non-heritable fluctuations. The mutation to full eye in the 36th generation is neglected in this consideration.

If the sib-matings are included, the changes in the general features of the range are the same (figure 99). The lowest individuals again follow the general course of the lowest one of the unselected population, but there are two marked projections downward which represent two very low individuals. These are probably ultra-bar mutants like that in the 2nd generation but which died without leaving offspring and therefore can not be certainly listed as ultra-bar. At the upper limit the lowering of range between the 12th and 14th generations may again be made out but its location is not as clear here as in the direct line.

In females of the low direct line (figure 100) the lower limit of the range jumps up from −9.68 to −5.60 during the first generation and then fluctuates around the latter value. The two downward extensions, one in the 24th and one in the 36th generation, come within the range of ultra-bar heterozygotes, but are not listed as ultra-bar as they left no offspring during the test. The upward jump in the first generation is due to the decrease in heterozygosis as previously explained and does not represent a true restriction of range except for that part of it which is due to the fact that the number of individuals in the selected generations is smaller than in the unselected parental population. That the decrease in heterozygosis and the resulting increase in female facet values is the principal element in the result is indicated by the fact that at the upper limit of the range in the high-selection line there is an extension rather than a restriction. The females therefore confirm the conclusion drawn from the male data that there is no change in the lower limit of range as a result of downward selection.

The upper limit in the females of the low direct line drops during the first generation from +8.75 to +2.36 and with considerable irregularity maintains the latter level for the first twelve generations. There is then a sudden drop to a level about four units lower than this. The fluctuation is now around a value of −1.50 until the end of the selection. The drop in this case is pronounced and, as already stated, undoubtedly represents a further step in the elimination of the factors for high facet number.

The less pronounced early drop in the upper limit in the females as compared with the males, is to be explained, as previously stated, by the rise in value of the females as a result of decrease in heterozygosis and does not indicate any difference between males and females in the essential sorting mechanism of selection.

When the sib-matings are included in the tabulation (figure 101) the range is extended somewhat because of the larger number of individuals, but all the features of the curve of range as described for the direct line can be made out.

In the high line the same principles apply as in the low line, but in the reverse direction as far as selection is concerned. In the males of the high direct line (figure 102), there is no upward extension of the original limit of +11.11 but rather a slight restriction which is probably the result of the smaller numbers of individuals in the single selection generations. As is to be expected on this view, individuals in some of the generations rise above the original value. At the lower limit the situation is quite different. The value rises from −10.72 to +0.77 in two generations and then fluctuates around zero until the 24th generation, when it goes up to a new level about three units above zero. In a very striking manner it is shown that there is an early elimination of factors for low facet numbers, followed by a considerable period of no progress and then by a small upward shift of about three units after the 24th generation, when apparently the masking effect of non-heritable fluctuations upon a small germinal factor was penetrated by selection.

Including the sib-matings (figure 103) the same general features can be made out for the range as in the direct line. High selection does not extend the range upward. Its whole effect is upon the lower limit.

The females of the high direct line (figure 104) during the first twenty selection generations have an upper limit of range slightly higher than that of the parental generation because of the rise in the value of all females due to the decrease in heterozygosis. Since a similar rise occurs in the low-selection lines, it can not be attributed to the upward selection. After the 20th generation there is a further rise which may be explained as due to the appearance of a sex-linked lethal factor affecting facet number in the viable heterozygous females. Such a lethal factor kills all males possessing it and all females with a double dose. Its existence is demonstrated by the fact that in the direct-line generations consisting of brother-and-sister matings there is a ratio of approximately two females to one male in generations 21 to 28. Since approximately half of the females in each generation have the new factor, it follows that the range is extended upward in the females but not in the males. After a few generations the lethal is lost because the females necessarily remain heterozygous for it and with the considerable non-heritable fluctuations in facet number, it follows that even with high selection sooner or later a female without the new factor is chosen and the factor is lost. The range then drops back to the level it had before the 20th generation.

At the lower limit the value rises from −9.68 to +3.01 in two generations and remains at the higher level until the 26th generation, when it reaches a new level at +6.00. This second rise corresponds to that in the males and represents a further sorting effect of the selection as already mentioned.

Including the sib-matings (figure 105) the higher limit shows the same features as in the direct line except for a slight extension of the range due to the greater number of individuals. At the lower limit the rapidity of the early elimination of the low individuals is not as great as in the direct line. At the new level the fluctuations are greater and the further rise to a higher level at the 26th generation is not as clear as in the direct line.

The data on range taken as a whole demonstrate in a striking manner the fact that the early pronounced effect of selection is not due to an extension of the range but to the elimination in the low line of those germinal factors that produce high facet number and in the high line of those that produce low facet numbers. This effect is rapidly accomplished and then the ranges remain fairly uniform except for one small shift in the low line and two in the high line. In the low line there is a further restriction of the upper limit after the 12th generation which is due to the capture in the selection net of a minor germinal factor for high facet number which had up to this time escaped because of the protection of non-heritable fluctuations. In the high line the extension of the range in the females after the 20th generation is probably due to the appearance of a sex-linked lethal mutation of small degree which took place in the direction of selection and was preserved, therefore, for a few generations. In the 25th generation the restriction of the lower limit of range is due to the elimination of one of the original germinal differences which up to this time had escaped the selection net.

Mean

The values in the direct line with their probable errors are given in tables 12 to 15. All of them without the probable errors are collected in table 24 which gives as well the differences between the high and low lines for each sex and also the average differences between the two lines. It may be well to recall that the low- and high-line values as given represent directly the departures in ten-percent factorial units from the mean of the unselected population which, it will be recalled, is used as the zero point or point of reference. The table includes the mean value in each generation of the female offspring for both high and low lines and the difference between the two. Then follow the same values for the males, and finally, in the last column, the average of the two differences for each generation, or the amount of divergence of high and low lines. This last value is the best expression for the effectiveness of the past selection at any point.

The values for the means including all matings are given in tables 16 to 19. The means for all matings in each generation are collected without their probable errors in table 25. The arrangement of the data is the same as that for the direct lines.

A comparison of the direct-line values with those of all matings can be made in 40 of the generations. In 31 of these the direct line has the greater average difference between high and low, while the averages of all matings are ahead in only 9. This may signify one or both of the two probabilities (1) that there is a germinal difference between the two groups, and (2) that the introduction of the element of selection on the basis of performance as mentioned on page 8 gives a false appearance of greater effectiveness of selection in the direct line. That the second is true is evident from the fact that during the generations during which selection has no effect, the direct line has the same preponderance over "all matings" that it does during the early generations when selection is obviously effective.

In the more detailed discussion of the means, it will be convenient to consider: first, the total divergence between the high and low lines; second, the contribution of each line to the total divergence; and third, the female and male means in each line.

Total divergence

The difference between the high and low line in each generation is given in table 24 and figure 106 for direct-line matings and in table 25 and figure 107 for all matings. There is a pronounced increase in divergence during the first five generations, amounting to +11.49 in the direct line and to +10.69 if the sib-matings are included. This period is followed by one with considerable fluctuation but no essential advance to the 25th generation, when there is a value of +12.18 in the direct line and +11.42 in "all matings." From the 25th to the 29th generations there is a distinct upward trend to +16.53 in the direct line and +15.14 in "all matings." After the 29th generation there is no further advance but even a slight regression though the level remains somewhat higher than it was before the 25th generation. Evidently the sorting out of major differences was completed within the first five generations

TABLE 24

Differences between the means of the high and the low lines in each generation. Direct lines.

GENERATION	FEMALES			MALES			AVERAGE DIFFERENCE
	High	Low	Difference	High	Low	Difference	
1	+ 3.03	−2.07	+ 5.10	−0.35	−4.85	+ 4.50	+ 4.80
2	+ 7.09	+1.29	+ 5.80	+3.33	−2.58	+ 5.91	+ 5.85
3	+ 6.87	−3.13	+10.00	+2.29	−8.30	+10.59	+10.29
4	+ 8.22	−1.03	+ 9.25	+3.71	−6.03	+ 9.74	+ 9.49
5	+ 8.22	−1.93	+10.15	+6.99	−5.85	+12.84	+11.49
6	+ 7.22	−1.63	+ 8.85	+3.23	−5.14	+ 8.37	+ 8.61
7	+ 6.30	−2.38	+ 8.68	+4.00	−5.24	+ 9.24	+ 8.96
8	+ 4.99	+0.08	+ 4.91	+3.04	−5.12	+ 8.16	+ 6.53
9	+ 7.84	−0.77	+ 8.61	+6.46	−6.20	+12.66	+10.64
10	+ 7.39	−0.83	+ 8.22	+5.44	−6.47	+11.91	+10.06
11	+ 7.28	−1.08	+ 8.36	+4.54	−5.03	+ 9.57	+ 8.96
12	+ 7.27	−1.98	+ 9.25	+6.43	−6.61	+13.04	+11.14
13	+ 6.81	−3.13	+ 9.94	+3.89	−8.05	+11.94	+10.94
14	+ 7.26	−3.93	+11.19	+4.16	−8.38	+12.54	+11.86
15	+ 6.86	−3.09	+ 9.95	+3.15	−7.95	+11.10	+10.52
16	+ 8.17	−2.76	+10.93	+4.21	−8.67	+12.88	+11.90
17	+ 6.65	−4.31	+10.96	+3.86	−9.30	+13.16	+12.06
18	+ 6.07	−3.20	+ 9.27	+4.24	−7.81	+12.05	+10.66
19	+ 6.32	−4.11	+10.43	+2.51	−9.45	+11.96	+11.19
20	+ 6.72	−4.04	+10.76	+3.05	−9.61	+12.66	+11.71
21	+ 7.47	−3.62	+11.09	+4.93	−9.15	+14.08	+12.58
22	+ 8.78	−2.72	+11.50	+3.45	−7.76	+11.21	+11.35
23		−3.55			−8.29		
24	+ 8.07	−4.87	+12.94	+3.82	−7.82	+11.64	+12.29
25	+ 8.89	−2.73	+11.62	+4.95	−7.80	+12.75	+12.18
26	+10.99	−3.06	+14.05	+5.44	−8.57	+14.01	+14.03
27	+11.02	−3.70	+14.72	+7.09	−8.68	+15.77	+15.24
28	+11.65	−3.59	+15.24	+6.77	−8.36	+15.13	+15.18
29	+12.37	−3.81	+16.18	+7.71	−9.18	+16.89	+16.53
30	+11.45	−3.69	+15.14	+6.73	−8.77	+15.50	+15.32
31	+ 9.43	−2.36	+11.79	+5.60	−7.41	+13.01	+12.40
32	+ 9.04	−1.89	+10.93	+4.38	−7.00	+11.38	+11.15
33	+ 9.72	−4.56	+14.28	+5.69	−8.71	+14.40	+14.34
34	+ 9.15	−4.01	+13.16	+5.70	−9.10	+14.80	+13.98
35	+ 9.21	−4.31	+13.52	+7.20	−7.68	+14.88	+14.20
36	+ 9.17	−3.57	+12.74	+6.27	−8.76	+15.03	+13.88
37	+10.74	−2.71	+13.45	+6.75	−6.73	+13.48	+13.46
38	+ 9.48	−2.64	+12.12	+5.15	−8.05	+13.20	+12.66
39	+ 8.77	−4.40	+13.17	+4.73	−7.99	+12.72	+12.94
40	+ 9.36	−3.36	+12.72	+5.44	−9.22	+14.66	+13.69
41	+10.28	−4.14	+14.42	+6.09	−9.31	+15.40	+14.91
42	+ 8.07	−2.84	+10.91	+5.19	−7.59	+12.78	+11.84

TABLE 25

Differences between the means of the high and the low lines in each generation. All matings.

GENERATION	FEMALES			MALES			AVERAGE DIFFERENCE
	High	Low	Difference	High	Low	Difference	
1	+ 2.04	−2.07	+ 4.11	−1.32	−4.85	+ 3.53	+ 3.82
2	+ 5.53	+0.73	+ 4.80	+1.19	−3.44	+ 4.63	+ 4.71
3	+ 5.76	−1.86	+ 7.62	+1.09	−6.35	+ 7.44	+ 7.53
4	+ 7.19	−1.03	+ 8.22	+2.79	−6.03	+ 8.82	+ 8.52
5	+ 8.13	−1.93	+10.06	+5.48	−5.85	+11.33	+10.69
6	+ 6.92	−1.61	+ 8.53	+3.24	−5.67	+ 8.91	+ 8.72
7	+ 5.34	−2.38	+ 7.72	+2.92	−5.24	+ 8.16	+ 7.94
8	+ 4.91	−0.19	+ 5.10	+2.66	−5.47	+ 8.13	+ 6.61
9	+ 6.79	−0.64	+ 7.43	+4.02	−6.08	+10.10	+ 8.76
10	+ 7.29	−0.30	+ 7.59	+4.81	−5.44	+10.25	+ 8.92
11	+ 6.63	−0.72	+ 7.35	+3.80	−4.85	+ 8.65	+ 8.00
12	+ 7.15	−1.82	+ 8.97	+5.77	−6.56	+12.33	+10.65
13	+ 6.81	−2.38	+ 9.19	+3.89	−6.62	+10.51	+ 9.85
14	+ 7.76	−2.86	+10.62	+5.06	−8.55	+13.61	+12.11
15	+ 6.69	−3.26	+ 9.95	+3.01	−7.96	+10.97	+10.46
16	+ 6.97	−2.86	+ 9.83	+3.65	−8.67	+12.32	+11.07
17	+ 6.65	−1.61	+ 8.26	+3.72	−7.12	+10.84	+ 9.55
18	+ 6.07	−2.87	+ 8.94	+4.24	−7.92	+12.16	+10.55
19	+ 6.32	−2.67	+ 8.99	+2.51	−7.98	+10.49	+ 9.74
20	+ 6.62	−2.81	+ 9.43	+3.16	−8.60	+11.76	+10.59
21	+ 7.60	−3.34	+10.94	+5.18	−9.13	+14.31	+12.62
22	+ 7.00	−2.54	+ 9.54	+4.60	−7.74	+12.34	+10.94
23	+ 6.91	−3.39	+10.30	+4.12	−8.09	+12.21	+11.25
24	+ 7.80	−4.45	+12.25	+4.65	−8.34	+12.99	+12.62
25	+ 8.01	−2.47	+10.48	+4.60	−7.76	+12.36	+11.42
26	+ 9.81	−2.55	+12.36	+5.50	−7.41	+12.91	+12.63
27	+ 9.47	−3.23	+12.70	+5.50	−8.60	+14.10	+13.40
28	+11.53	−3.41	+14.94	+6.48	−8.23	+14.71	+14.82
29	+11.76	−3.24	+15.00	+6.86	−8.42	+15.28	+15.14
30	+10.91	−3.51	+14.42	+6.11	−8.74	+14.85	+14.63
31	+ 9.82	−2.79	+12.61	+5.49	−7.68	+13.17	+12.89
32	+ 9.62	−2.62	+12.24	+5.10	−8.05	+13.15	+12.69
33	+ 9.24	−4.33	+13.57	+4.90	−8.90	+13.80	+13.68
34	+ 8.33	−3.93	+12.26	+4.72	−9.05	+13.77	+13.01
35	+ 8.27	−3.62	+11.89	+4.83	−7.90	+12.73	+12.31
36	+ 8.35	−3.80	+12.15	+4.81	−9.06	+13.87	+13.01
37	+10.09	−2.85	+12.94	+6.41	−7.36	+13.77	+13.35
38	+ 9.47	−2.64	+12.11	+5.36	−8.05	+13.41	+12.76
39	+ 9.31	−3.86	+13.17	+5.22	−8.23	+13.45	+13.31
40	+ 8.91	−2.81	+11.72	+5.11	−8.37	+13.48	+12.60
41	+10.40	−3.80	+14.20	+6.14	−9.15	+15.29	+14.74
42	+ 8.07	−2.84	+10.91	+5.19	−7.59	+12.78	+11.84

or earlier. The later effect as shown elsewhere is due either to the elimination of smaller germinal differences which had previously been masked by non-heritable factors or to the appearance of new mutations of small degree.

The contributions of low and high lines to the total divergence

These are shown in figures 108 and 109 and in the fourth columns of tables 26 to 29 where the female and male means are combined. It will be noticed that in the low line the initial period of pronounced sorting

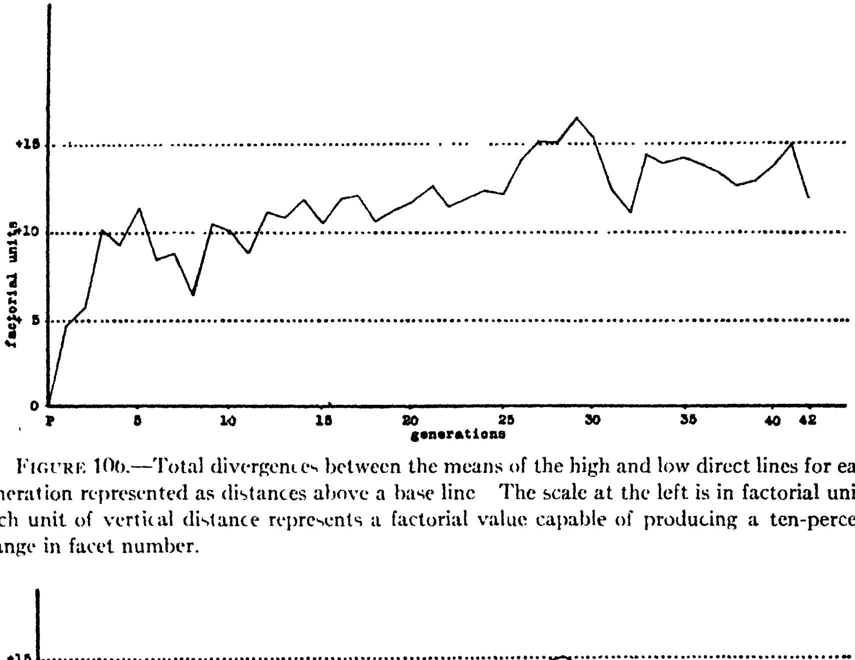


FIGURE 106.—Total divergences between the means of the high and low direct lines for each generation represented as distances above a base line The scale at the left is in factorial units. Each unit of vertical distance represents a factorial value capable of producing a ten-percent change in facet number.

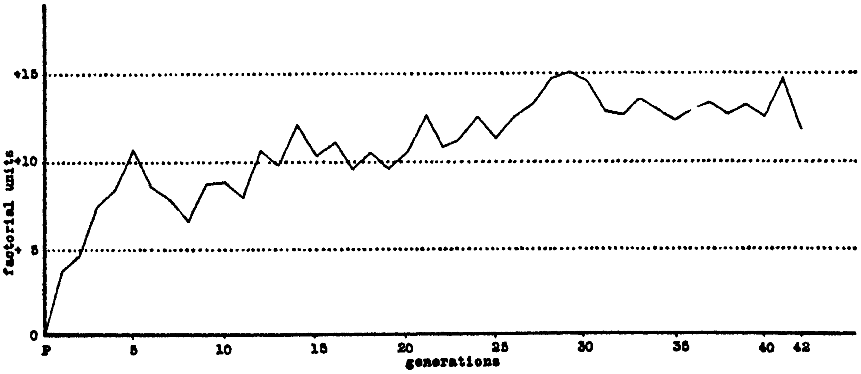


FIGURE 107.—Total divergences between the means of the high and low lines including all matings for each generation. Arrangement as in figure 106.

effect is completed by the first selection in which the value decreases to −3.46. From that time until the 11th generation, when the value is −3.05 in the direct line and −2.78 in "all matings," there is no further decrease. From the 11th to the 14th generations there is a rapid decrease to a new level, the value at the 14th generation being −6.15 in the direct line and −5.70 in "all matings." After the 14th generation there is no further effect of selection, the values fluctuating around −6.00.

In the high line the initial period of effectiveness is completed at the fifth generation when the mean has reached a value of +7.60 in the direct line and +6.80 in "all matings." From that time there is no further increase until the 20th generation has been passed, when the values increase until the 28th generation. Following the 28th there is a decline, but the level remains somewhat higher than it was before the 20th generation. The rise between the 20th and 28th generations is due to the appearance of a sex-linked lethal factor, the following drop to the elimination of the lethal and the retention of a somewhat higher level to the elimination of a germinal factor for low facet number which took place following the 25th generation.

Returning to the divergence between the two lines, it is clear that the general upward trend from the 8th to the 29th generations shown in figures 106 and 107 is due on the one hand to the elimination of a high factor in the low line between the 12th and 14th generations, and to the two processes described above as taking place in the high line between the 20th and 29th generations. In fact a close examination of the upward trend between the 8th and 29th generations shows that between the 14th generation, when the first-mentioned elimination takes place, and the 20th, when the lethal factor appears, there is actually no increase in divergence.

The female and male means

The female and male means are considered separately in figures 110 and 111 for the direct line and figures 112 and 113 for "all matings." These figures show in a very striking manner the great decrease in sexual dimorphism during the course of selection. The males show a much lower value comparatively than the females in both high and low lines. This gives the impression that selection in the high line is much more effective in its action upon the females than upon the males, and that the reverse is true for the low line where the effectiveness seems to be largely confined to the males. This appearance obviously is due to the

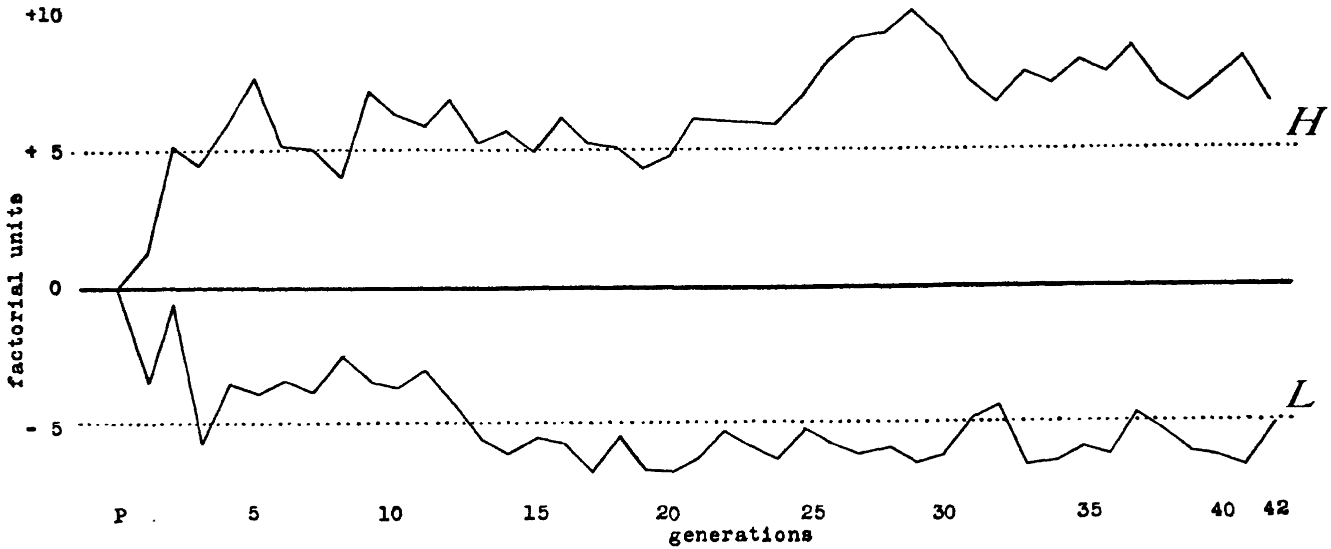


Figure 108.—The means of the high direct line (H) and low direct line (L) compared with the mean of the unselected population (zero) for each selection generation. The female and male values are averaged in each case. The present figure gives the contribution of each of the two lines to the total divergence. The vertical distance between the upper zigzag line and the lower zigzag line in each generation gives the corresponding total divergence of figure 106.

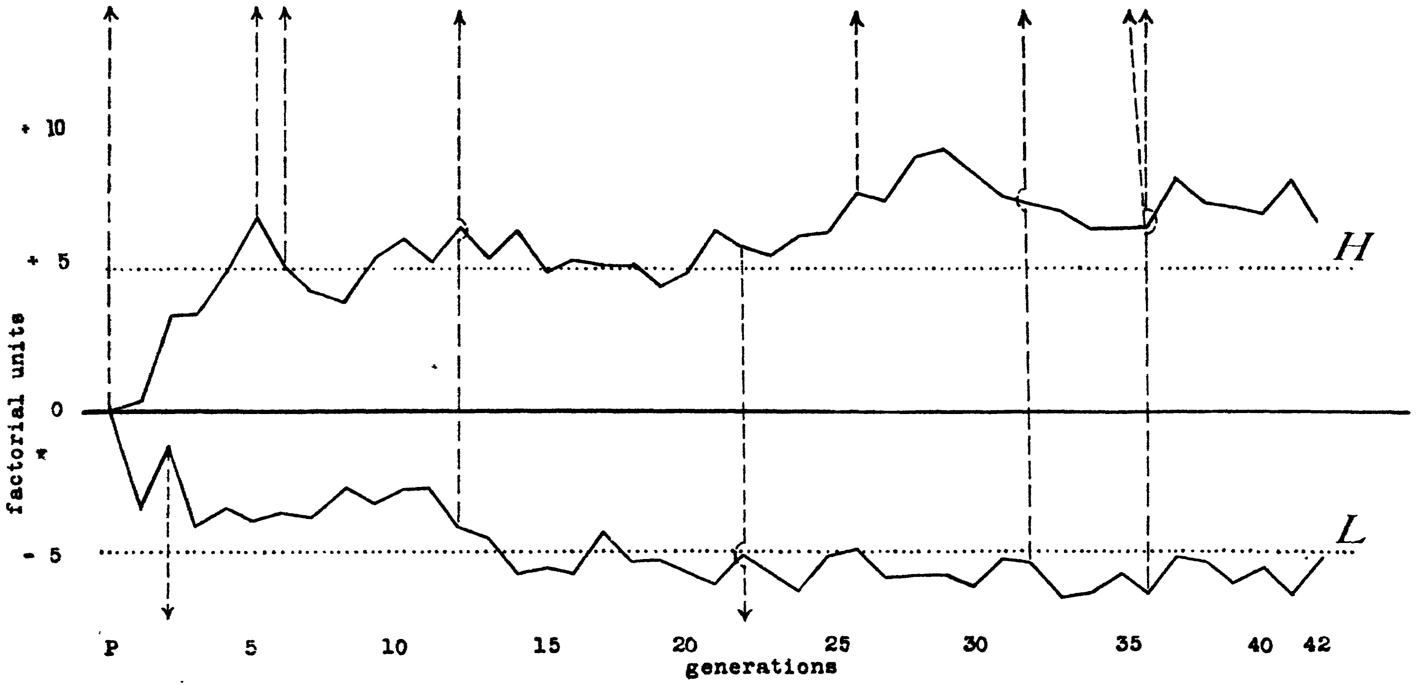


FIGURE 109.—The means of the high line including all matings (H) and low line including all matings (L) compared with the mean of the unselected population (zero) for each selection generation. Arrangement as in figure 108. The vertical distance between H and L in each generation gives the corresponding total divergence of figure 107.

TABLE 26

Degree of selection, difference between parents and offspring, and effectiveness of selection for each generation. Low line. Direct. Values in ten-percent factorial units.

GENERATION	WEIGHTED AVERAGE OF SELECTED PAIRS S	AVERAGE OF ♀ AND ♂ MEANS PARENTAL GENERATION P	AVERAGE OF ♀ AND ♂ MEANS OFFSPRING O	DEGREE OF SELECTION S−P	DIFFERENCE BETWEEN PARENTS AND OFFSPRING O−	EFFECTIVENESS OF SELECTION O−P
1	−5.41	0.00	−3.46	−5.41	+1.95	−3.46
2	−8.15	−3.46	−0.64	−4.69	+7.51	+2.82
3	−4.99	−0.64	−5.71	−4.35	−0.72	−5.07
4	−7.46	−5.71	−3.53	−1.75	+3.93	+2.18
5	−3.53	−3.53	−3.89	0.00	−0.36	−0.36
6	−4.80	−3.89	−3.38	−0.91	+1.42	+0.51
7	−6.36	−3.38	−3.81	−2.98	+3.38	−0.43
8	−6.59	−3.81	−2.52	−2.78	+4.07	+1.29
9	−3.45	−2.52	−3.48	−0.93	−0.03	−0.96
10	−2.96	−3.48	−3.65	+0.52	−0.69	−0.17
11	−6.36	−3.65	−3.05	−2.71	+3.31	+0.60
12	−6.48	−3.05	−4.29	−3.43	+2.19	−1.24
13	−7.21	−4.29	−5.59	−2.92	+1.62	−1.30
14	−6.61	−5.59	−6.15	−1.02	+0.46	−0.56
15	−6.61	−6.15	−5.52	−0.46	+1.09	+0.63
16	−8.16	−5.52	−5.71	−2.64	+2.45	−0.19
17	−9.15	−5.71	−6.80	−3.44	+2.35	−1.09
18	−7.13	−6.80	−5.50	−0.33	+1.63	+1.30
19	−6.00	−5.50	−6.78	−0.50	−0.78	−1.28
20	−8.82	−6.78	−6.82	−2.04	+2.00	−0.04
21	−6.48	−6.82	−6.38	+0.34	+0.10	+0.44
22	−6.96	−6.38	−5.24	−0.58	+1.72	+1.14
23	−6.23	−5.24	−5.92	−0.99	+0.31	−0.68
24	−8.34	−5.92	−6.34	−2.42	+2.00	−0.42
25	−8.92	−6.34	−5.26	−2.58	+3.66	+1.08
26	−5.40	−5.26	−5.81	−0.14	−0.41	−0.55
27	−5.75	−5.81	−6.19	+0.06	−0.44	−0.38
28	−6.11	−6.19	−5.97	+0.08	+0.14	+0.22
29	−8.34	−5.97	−6.49	−2.37	+1.85	−0.52
30	−7.88	−6.49	−6.23	−1.39	+1.65	+0.26
31	−7.59	−6.23	−4.88	−1.36	+2.71	+1.35
32	−5.97	−4.88	−4.44	−1.09	+1.53	+0.44
33	−7.23	−4.44	−6.63	−2.79	+0.60	−2.19
34	−7.42	−6.63	−6.55	−0.79	+0.87	+0.08
35	−7.55	−6.55	−5.99	−1.00	+1.56	+0.56
36	−7.46	−5.99	−6.16	−1.47	+1.30	−0.17
37	−6.92	−6.16	−4.72	−0.76	+2.20	+1.44
38	−7.34	−4.72	−5.34	−2.62	+2.00	−0.62
39	−7.51	−5.34	−6.19	−2.17	+1.41	−0.85
40	−9.49	−6.19	−6.29	−3.30	+3.20	−0.10
41	−6.23	−6.29	−6.72	+0.06	−0.49	−0.43
42	−7.67	−6.72	−5.21	−0.95	+2.46	+1.51
Average				−1.71		−0.12

TABLE 27

Degree of selection, difference between parents and offspring and effectiveness of selection for each generation. Low line. All matings. Values in ten-percent factorial units.

GENERATION	WEIGHTED AVERAGE OF SELECTED PAIRS S	AVERAGE OF ♀ AND ♂ MEANS PARENTAL GENERATION P	AVERAGE OF ♀ AND ♂ MEANS OFFSPRING O	DEGREE OF SELECTION S−P	DIFFERENCE BETWEEN PARENTS AND OFFSPRING O−S	EFFECTIVENESS OF SELECTION O−P
1	−6.09	0.00	−3.46	−6.09	+2.63	−3.46
2	−6.55	−3.46	−1.35	−3.09	+5.20	+2.11
3	−5.30	−1.35	−4.10	−3.95	+1.20	−2.75
4	−7.46	−4.10	−3.53	−3.36	+3.93	+0.57
5	−3.53	−3.53	−3.89	0.00	−0.36	−0.36
6	−5.52	−3.89	−3.64	−1.63	+1.88	+0.25
7	−6.36	−3.64	−3.81	−2.72	+2.55	−0.17
8	−5.88	−3.81	−2.83	−2.07	+3.05	+0.98
9	−3.84	−2.83	−3.36	−1.01	+0.48	−0.53
10	−3.35	−3.36	−2.87	+0.01	+0.48	+0.49
11	−6.11	−2.87	−2.78	−3.24	+3.33	+0.09
12	−6.35	−2.78	−4.19	−3.57	+2.16	−1.41
13	−6.73	−4.19	−4.50	−2.54	+2.23	−0.31
14	−6.95	−4.50	−5.70	−2.45	+1.25	−1.20
15	−6.64	−5.70	−5.61	−0.94	+1.03	+0.09
16	−7.97	−5.61	−5.76	−2.36	+2.21	−0.15
17	−7.85	−5.76	−4.36	−2.09	+3.49	+1.40
18	−6.98	−4.36	−5.39	−2.62	+1.59	−1.03
19	−5.96	−5.39	−5.32	−0.57	+0.64	+0.07
20	−7.14	−5.32	−5.70	−1.82	+1.44	−0.38
21	−6.96	−5.70	−6.23	−1.26	+0.73	−0.53
22	−7.14	−6.23	−5.14	−0.91	+2.00	+1.09
23	−5.79	−5.14	−5.74	−0.65	+0.05	−0.60
24	−8.41	−5.74	−6.39	−2.67	+2.02	−0.65
25	−8.55	−6.39	−5.11	−2.16	+3.44	+1.28
26	−5.78	−5.11	−4.98	−0.67	+0.80	+0.13
27	−6.35	−4.98	−5.91	−1.37	+0.44	−0.93
28	−6.40	−5.91	−5.82	−0.49	+0.58	+0.09
29	−7.92	−5.82	−5.83	−2.10	+2.09	−0.01
30	−7.59	−5.83	−6.12	−1.76	+1.47	−0.29
31	−7.35	−6.12	−5.23	−1.23	+2.12	+0.89
32	−5.98	−5.23	−5.33	−0.75	+0.65	−0.10
33	−7.29	−5.33	−6.61	−1.96	+0.68	−1.28
34	−7.48	−6.61	−6.49	−0.87	+0.99	+0.12
35	−6.91	−6.49	−5.76	−0.42	+1.15	+0.73
36	−7.64	−5.76	−6.43	−1.88	+1.21	−0.67
37	−7.25	−6.43	−5.10	−0.82	+2.15	+1.33
38	−7.34	−5.10	−5.34	−2.24	+2.00	−0.24
39	−7.09	−5.34	−6.04	−1.75	+1.05	−0.70
40	−6.90	−6.04	−5.59	−0.86	+1.31	+0.45
41	−6.52	−5.59	−6.47	−0.93	+0.05	−0.88
42	−7.68	−6.47	−5.21	−1.21	+2.47	+1.26
Average				−1.79		−0.12

TABLE 28

Degree of selection, difference between parents and offspring and effectiveness of selection for each generation. High line. Direct. Values in ten-percent factorial units.

GENERATION	WEIGHTED AVERAGE OF SELECTED PAIRS S	AVERAGE OF ♀ AND ♂ MEANS PARENTAL GENERATION P	AVERAGE OF ♀ AND ♂ MEANS OFFSPRING O	DEGREE OF SELECTION S−P	DIFFERENCE BETWEEN PARENTS AND OFFSPRING O−S	EFFECTIVENESS OF SELECTION O−P
1	+ 7.67	0.00	+ 1.34	+7.67	−6.33	+1.34
2	+ 7.18	+ 1.34	+ 5.21	+5.84	−1.97	+3.87
3	+ 7.00	+ 5.21	+ 4.58	+1.79	−2.42	−0.63
4	+ 5.89	+ 4.58	+ 5.96	+1.31	+0.07	+1.38
5	+10.21	+ 5.96	+ 7.60	+4.25	−2.61	+1.64
6	+ 7.24	+ 7.60	+ 5.22	−0.36	−2.02	−2.38
7	+ 6.97	+ 5.22	+ 5.15	+1.75	−1.82	−0.07
8	+ 7.52	+ 5.15	+ 4.01	+2.37	−3.51	−1.14
9	+ 6.52	+ 4.01	+ 7.15	+2.51	+0.63	+3.14
10	+ 9.93	+ 7.15	+ 6.41	+2.78	−3.52	−0.74
11	+ 7.22	+ 6.41	+ 5.91	+0.81	−1.31	−0.50
12	+ 6.84	+ 5.91	+ 6.85	+0.93	+0.01	+0.94
13	+ 9.31	+ 6.85	+ 5.35	+2.46	−3.96	−1.50
14	+ 7.62	+ 5.35	+ 5.71	+2.27	−1.91	+0.36
15	+ 7.76	+ 5.71	+ 5.00	+2.05	−2.76	−0.71
16	+ 6.32	+ 5.00	+ 6.19	+1.32	−0.13	+1.19
17	+10.25	+ 6.19	+ 5.25	+4.06	−5.00	−0.94
18	+ 1.99	+ 5.25	+ 5.15	−3.26	+3.16	−0.10
19	+ 7.43	+ 5.15	+ 4.41	+2.28	−3.02	−0.74
20	+ 3.94	+ 4.41	+ 4.88	−0.47	+0.94	+0.47
21	+ 7.72	+ 4.88	+ 6.20	+2.84	−1.51	+1.33
22	+10.42	+ 6.20	+ 6.12	+4.22	−4.30	−0.08
23						
24	+ 9.59	+ 6.12	+ 5.94	+3.47	−3.65	−0.18
25	+11.16	+ 5.94	+ 6.92	+5.22	−4.24	+0.98
26	+10.13	+ 6.92	+ 8.21	+3.21	−1.92	+1.29
27	+10.09	+ 8.21	+ 9.05	+1.88	−1.04	+0.84
28	+ 9.98	+ 9.05	+ 9.21	+0.93	−0.77	+0.16
29	+12.78	+ 9.21	+10.04	+3.57	−2.74	+0.83
30	+11.99	+10.04	+ 9.09	+1.95	−2.90	−0.95
31	+10.59	+ 9.09	+ 7.51	+1.50	−3.08	−1.58
32	+ 9.32	+ 7.51	+ 6.71	+1.81	−2.61	−0.80
33	+ 7.43	+ 6.71	+ 7.70	+0.72	+0.27	+0.99
34	+10.98	+ 7.70	+ 7.42	+3.28	−3.56	−0.28
35	+ 8.32	+ 7.42	+ 8.20	+0.90	−0.12	+0.78
36	+10.01	+ 8.20	+ 7.72	+1.81	−2.29	−0.48
37	+ 8.92	+ 7.72	+ 8.74	+1.20	−0.18	+1.02
38	+ 9.22	+ 8.74	+ 7.31	+0.48	−1.91	−1.43
39	+10.46	+ 7.31	+ 6.75	+3.15	−3.71	−0.56
40	+ 9.11	+ 6.75	+ 7.40	+2.36	−1.71	+0.65
41	+ 8.51	+ 7.40	+ 8.18	+1.11	−0.33	+0.78
42	+ 8.83	+ 8.18	+ 6.63	+0.65	−2.20	−1.55
Average				+2.16		+0.16

TABLE 29

Degree of selection, difference between parents and offspring and effectiveness of selection for each generation. High line. All matings. Values in ten-percent factorial units.

GENERATION	WEIGHTED AVERAGE OF SELECTED PAIRS S	AVERAGE OF ♀ AND ♂ MEANS PARENTAL GENERATION P	AVERAGE OF ♀ AND ♂ MEANS OFFSPRING O	DEGREE OF SELECTION S−P	DIFFERENCE BETWEEN PARENTS AND OFFSPRING O−S	EFFECTIVENESS OF SELECTION O−P
1	+ 7.33	0.00	+0.36	+7.33	−6.97	+0.36
2	+ 4.38	+0.36	+3.36	+4.02	−1.02	+3.00
3	+ 6.97	+3.36	+3.42	+3.61	−3.55	+0.06
4	+ 5.98	+3.42	+4.99	+2.56	−0.99	+1.57
5	+ 8.80	+4.99	+6.80	+3.81	−2.00	+1.81
6	+ 7.57	+6.80	+5.08	+0.77	−2.49	−1.72
7	+ 6.94	+5.08	+4.13	+1.86	−2.81	−0.95
8	+ 7.53	+4.13	+3.78	+3.40	−3.75	−0.35
9	+ 5.54	+3.78	+5.40	+1.76	−0.14	+1.62
10	+ 9.69	+5.40	+6.05	+4.29	−3.64	+0.65
11	+ 7.85	+6.05	+5.21	+1.80	−2.64	−0.84
12	+ 6.57	+5.21	+6.46	+1.36	−0.11	+1.25
13	+ 9.31	+6.46	+5.35	+2.85	−3.96	−1.11
14	+ 7.77	+5.35	+6.41	+2.42	−1.36	+1.06
15	+ 7.80	+6.41	+4.85	+1.39	−2.95	−1.56
16	+ 6.31	+4.85	+5.31	+1.46	−1.00	+0.46
17	+ 8.75	+5.31	+5.18	+3.44	−3.57	−0.13
18	+ 1.99	+5.18	+5.15	−3.19	+3.16	−0.03
19	+ 7.44	+5.15	+4.41	+2.29	−3.03	−0.74
20	+ 4.09	+44.1	+4.89	−0.32	+0.80	+0.48
21	+ 7.74	+4.89	+6.39	+2.85	−1.35	+1.50
22	+ 8.03	+6.39	+5.80	+1.64	−2.23	−0.59
23	+ 7.66	+5.80	+5.51	+1.86	−2.15	−0.29
24	+ 7.03	+5.51	+6.22	+1.52	−0.81	+0.71
25	+ 9.61	+6.22	+6.30	+3.39	−3.31	+0.08
26	+ 9.70	+6.30	+7.65	+3.40	−2.05	+1.35
27	+10.01	+7.65	+7.48	+2.36	−2.53	−0.17
28	+10.07	+7.48	+9.00	+2.59	−1.07	+1.52
29	+10.24	+9.00	+9.31	+1.24	−0.93	+0.31
30	+11.50	+9.31	+8.51	+2.19	−2.99	−0.80
31	+10.59	+8.51	+7.65	+2.08	−2.94	−0.86
32	+ 8.59	+7.65	+7.36	+0.94	−1.23	−0.29
33	+ 9.33	+7.36	+7.07	+1.97	−2.26	−0.29
34	+ 8.68	+7.07	+6.52	+1.61	−2.16	−0.55
35	+ 7.21	+6.52	+6.55	+0.69	−0.66	+0.03
36	+ 9.43	+6.55	+6.58	+2.88	−2.85	+0.03
37	+ 8.41	+6.58	+8.25	+1.83	−0.16	+1.67
38	+ 8.97	+8.25	+7.41	+0.72	−1.56	−0.84
39	+ 8.94	+7.41	+7.26	+1.53	−1.68	−0.15
40	+ 9.28	+7.26	+7.01	+2.02	−2.27	−0.25
41	+ 8.58	+7.01	+8.27	+1.57	−0.31	+1.26
42	+ 8.83	+8.27	+6.63	+0.56	−2.20	−1.64
Average				+2.10		+0.16

decrease in sexual dimorphism, the graphs being constructed on the basis of the departures of each sex from its mean in the unselected population.

Recognizing this peculiarity in the graphs for individual sexes, an examination shows the same effective periods as in the previous groupings of the data. In both males and females, there is the same early effective period and the further ineffectiveness except during a single limited period in the low line and during the two overlapping periods in the high line.

The fluctuations during individual generations have many interesting features, but a discussion of them is purposely omitted at present as a further investigation of the environmental factors to which they seem to be due is under way.

Standard deviation

That the selection effect is the result of a sorting of original germinal differences is further shown by the decrease in variability as indicated by the standard deviations. The data are given in the last columns of tables 12 to 15 for the direct line and tables 16 to 19 for "all matings." Figures 114 to 121 give the same data in graphic form. The small numbers of individuals in many of the direct-line generations make these determinations less reliable than those for "all matings" but they are interesting for comparison with the latter. Though the fluctuations are much greater, the general trend of the curve of variability is much the same.

Corresponding to the marked effectiveness of selection on the range and mean during the early generations, there is a marked decrease in variability. The standard deviation drops from 3.12 ten-percent factorial units in the unselected females to 1.53 for "all matings" at the 3rd generation of the low line (figures 114 and 115). Among the males there is a corresponding drop from 3.91 to 1.70 in the fourth generation (figures 116 and 117).

In the high line the females drop to 1.24 in the 3rd generation of the direct line (figure 118) and to 1.95 in the same generation of "all matings" (figure 119). In the males the corresponding values are 1.53 for the 3rd generation of the direct line (figure 120) and 2.00 for the 4th generation of "all matings" (figure 121).

The changes in the range and mean following the 11th or 12th generation in the low line are reflected in a further decline in the standard

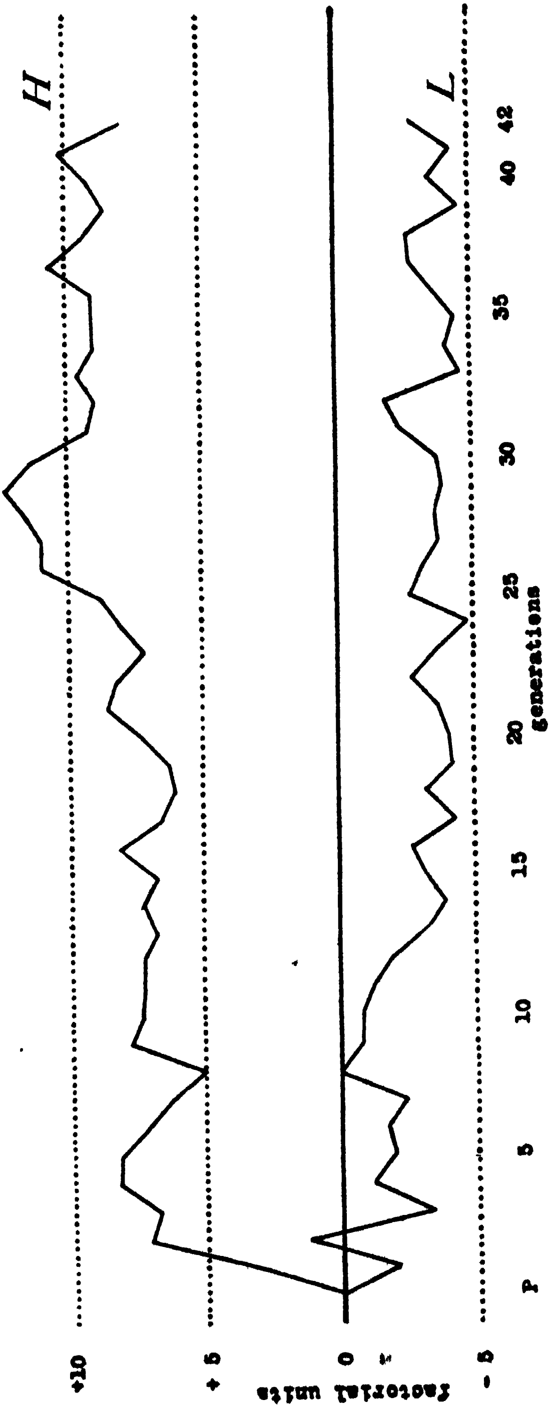


FIGURE 110.—Divergences of female means including all matings.

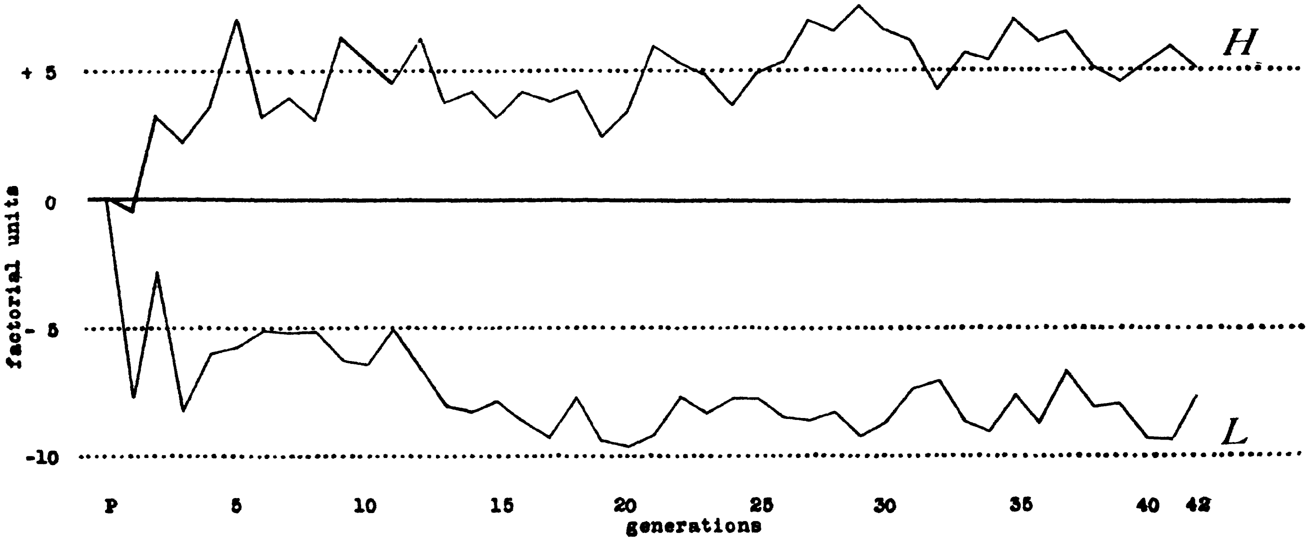


FIGURE 111.—Divergences of male means in the high direct line (H) and low direct line (L) from the mean of the unselected population (O) in each selection generation.

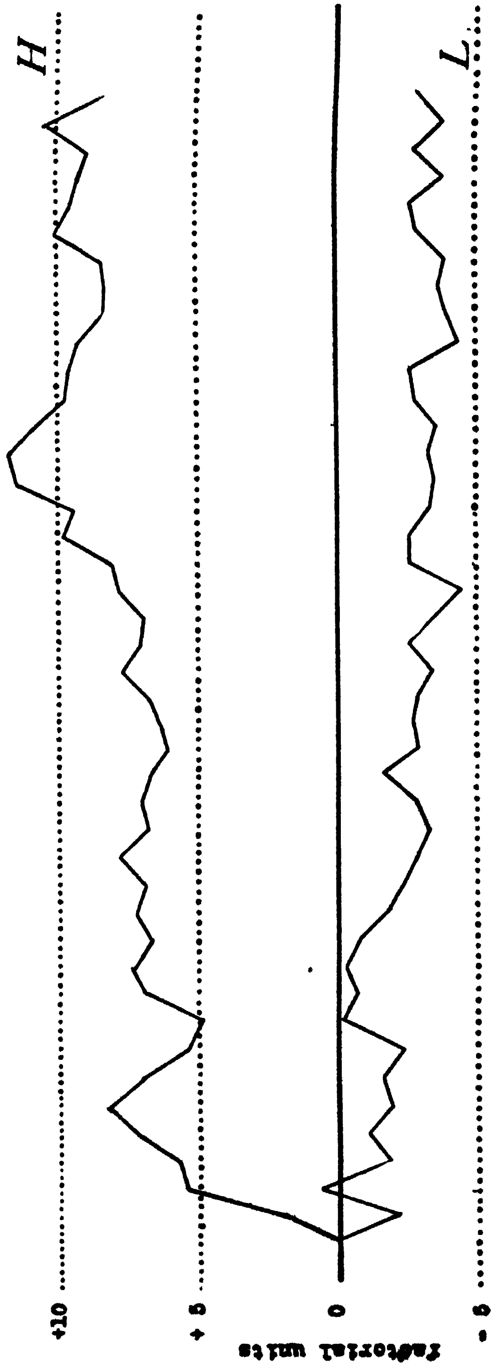


FIGURE 112.—Divergences of female means in the high direct line (H) and low direct line (L) from the mean of the unselected population (O) in each selection generation.

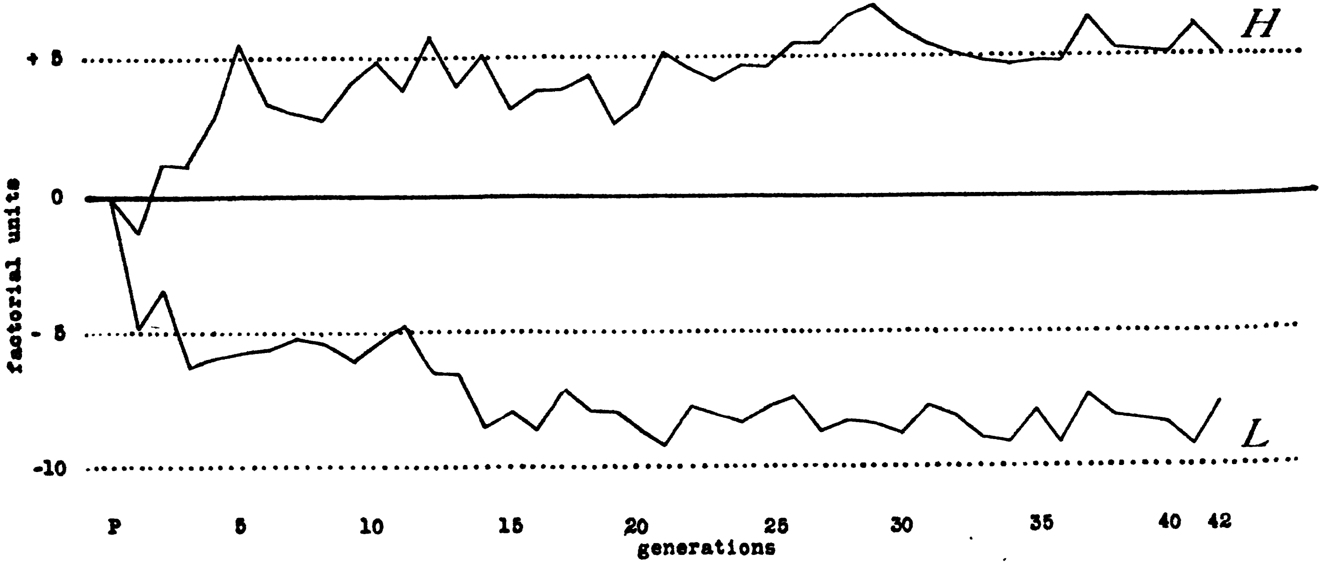


FIGURE 113.—Divergences of male means including all matings.

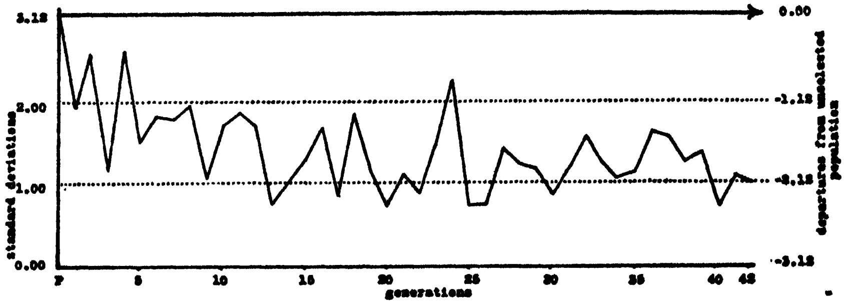


FIGURE 114.—Standard deviations of low-line direct females in factorial units which serve directly as coefficients of variation. The upper horizontal line represents the value of the standard deviation of the females in the unselected population. The bottom horizontal line represents zero standard deviation. The scale at the left gives the standard deviations and the scale at the right the decrease from the value of the unselected population.

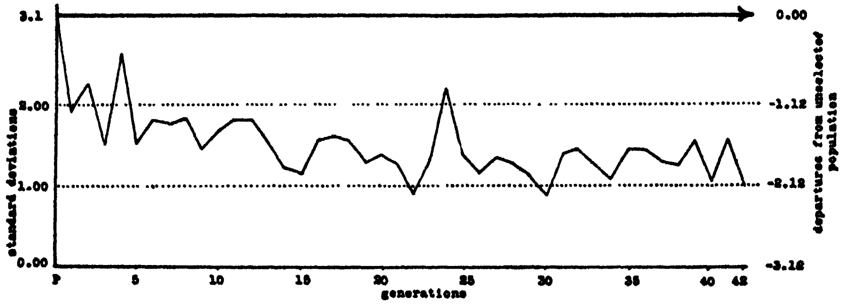


FIGURE 115.—Standard deviations of low-line females including all matings.

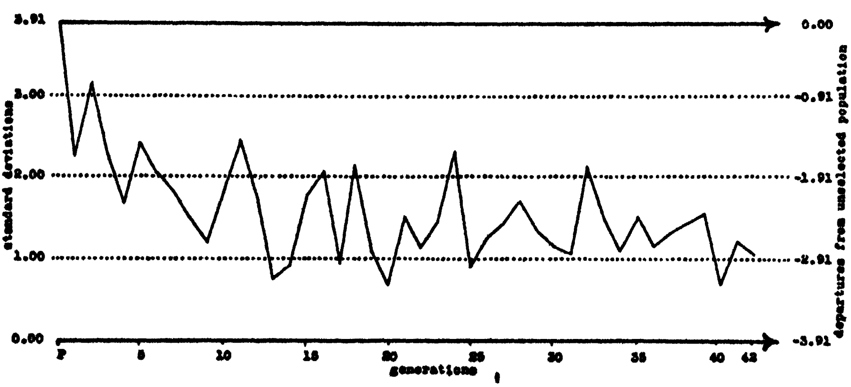


FIGURE 116.—Standard deviations of low-line direct males.

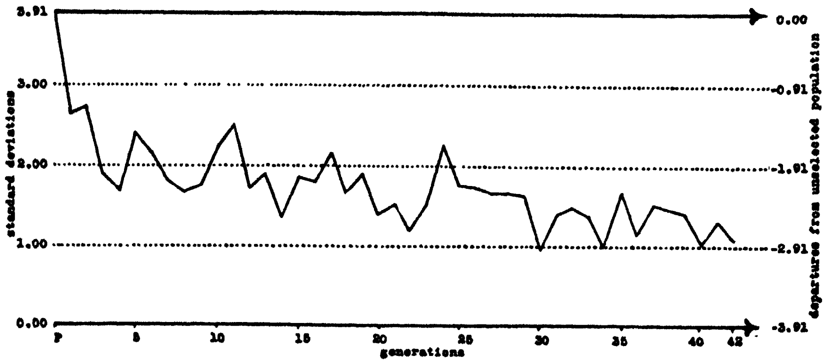


FIGURE 117.—Standard deviations of low-line males including all matings.

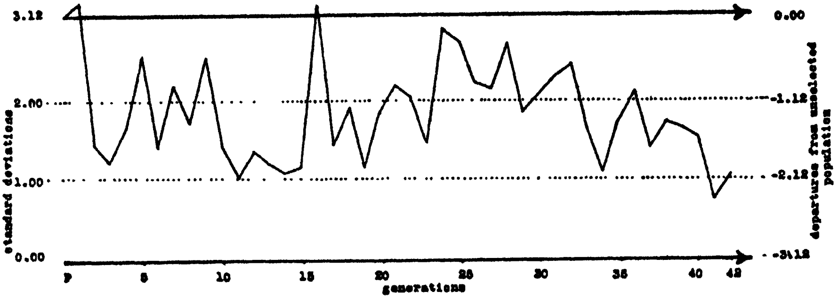


FIGURE 118.—Standard deviations of high-line direct females.

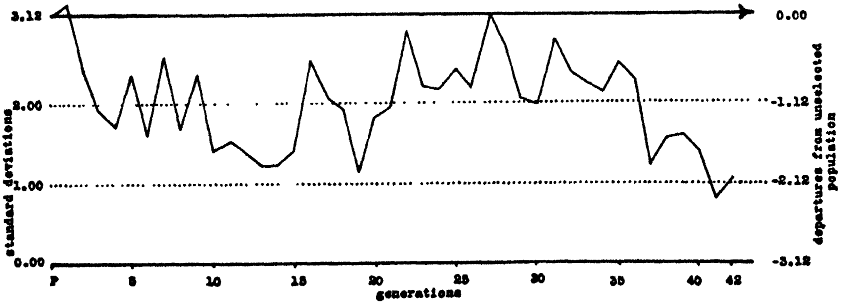


FIGURE 119.—Standard deviations of high-line females including all matings.

deviation at about this time. The decline can not be localized in a specific generation, but it is clear that in the generations following the 12th the standard deviations are at a slightly lower level than before. This confirms the view that the change is due to the elimination of a high-facet group rather than the addition of a low-facet one.

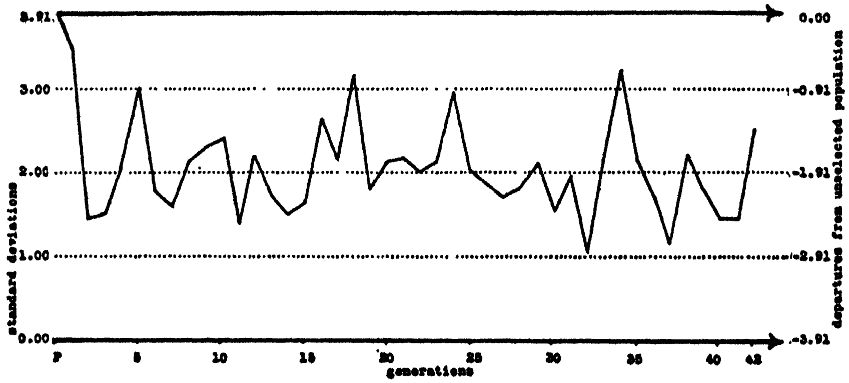


FIGURE 120.—Standard deviations of high-line direct males.

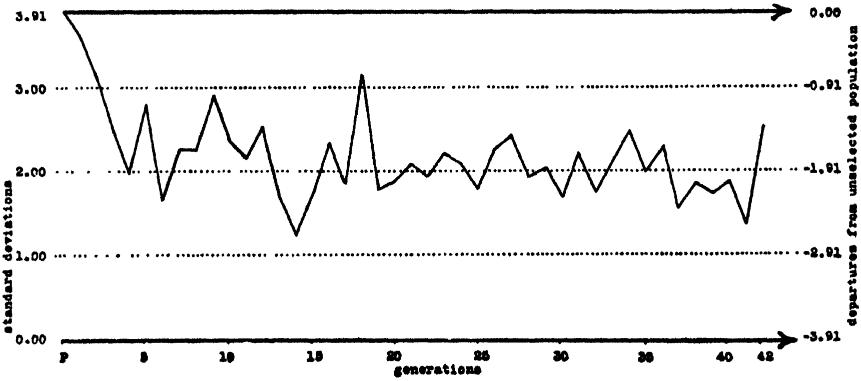


FIGURE 121.—Standard deviations of high-line males including all matings.

In the high line, the increase in the mean following the 20th generation and the change in range at the same time are reflected in an increase in the variability of the females but there is no change in the males. Later the female variability drops back to its previous level.

On the whole, the changes in variability confirm the general conclusion reached from a study of the ranges and means. Selection acts by eliminating certain germinal groups, thereby decreasing the variability. The only demonstrated new germinal difference during the course of the selection, apart from full eye and ultra-bar eye, is that which appears following the 20th generation of the high line. It is a sex-linked lethal and therefore affects the facet numbers of the females only.

Correlation between degree of selection and effectiveness of selection

While an effort was made in each generation to select the lowest possible pairs in the high line, the attempt was not always equally successful. It is, therefore, desirable to get in each generation an expression for the degree of selection (S—P) or the departure of the mean value of the selected pair (S) from the mean value of the parental generation (P) from which it was derived. This value should be minus in the low-selection lines and plus in the high lines. There may also be obtained a value for the difference between the mean of the parents and the mean of the offspring (O—S) in which O is the mean value of the offspring. Finally, these values may be compared with the effectiveness of the selection in each generation (O—P).

Tables 26 to 29 give the value in each generation of offspring of the mean of the selected pair (S) that gave rise to that generation, the mean of the parental generation (P) and the mean of the generation of offspring (O). Then follow the comparisons including a column for the values of the degree of selection (S—P), one for the difference between parents and offspring (O—S) and finally one for the effectiveness of selection (O—P). Table 26 and figure 122 give these values for the direct matings of the low line and table 28 and figure 124 for the direct matings of the high line. Table 27 and figure 123, and table 29 and figure 125 give the corresponding values for "all matings."

In figures 122 to 125 the vertical distances represent the degrees of selection in departures from the means of the parental generations, using the scale of factorial units. It will be noted that there are a few high selections in the low line and a few low selections in the high line. The horizontal distances represent increases in facet means of the offspring, as compared with the parental generation, when to the right of the axis of reference, and decreases when to the left. The length of the double-headed arrow between the horizontal axis of reference and the horizontal dotted line represents the average degree of selection for all the generations. The short double-headed arrow between the vertical axis and the vertical dotted line shows the corresponding average effectiveness of selection.

If all variation in facet number were due to fixed hereditary factors, it would be expected that there would be a complete correspondence between degree and effectiveness of selection. It is evident that there is no striking agreement between the two. Taking the direct matings, in the low line 37 of the 42 pairs have minus values and 20 of the 37 generations of offspring are lower than the parental generation and 17 are higher. In the high line 38 of the 41 pairs have a plus value and exactly half of the generations of offspring are higher than the parental generations while the other half are lower. It is obvious that the net effectiveness of selection is due to pronounced progress in a few generations.

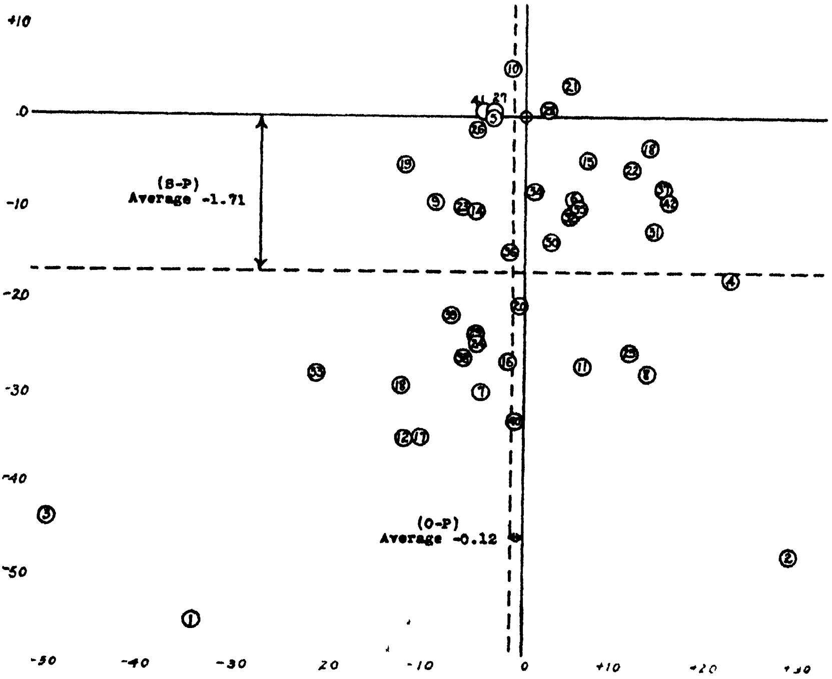


FIGURE 122.—Low direct line. Correlation between degree of selection (S — P) and departure of offspring from the mean of their parental generation (O — P). S = average of the means of the parents. P = mean of the parental generation, average of females and males. O = mean of offspring, average of females and males. Ordinates represent degree of selection. The unbroken horizontal line is the axis of reference, and the departures of parents from the means of their generations are put above or below it according as they are plus or minus. Abscissae represent effectiveness of selection, increases of mean of offspring over mean of the parental generation being to the right of the vertical unbroken line and decreases to the left. The dotted horizontal line gives the average degree of selection, i.e., the average departure of the selected pairs from the means of their generations. The vertical dotted line gives the average effectiveness of selection, i.e., the average departure of offspring from the means of their parental generations.

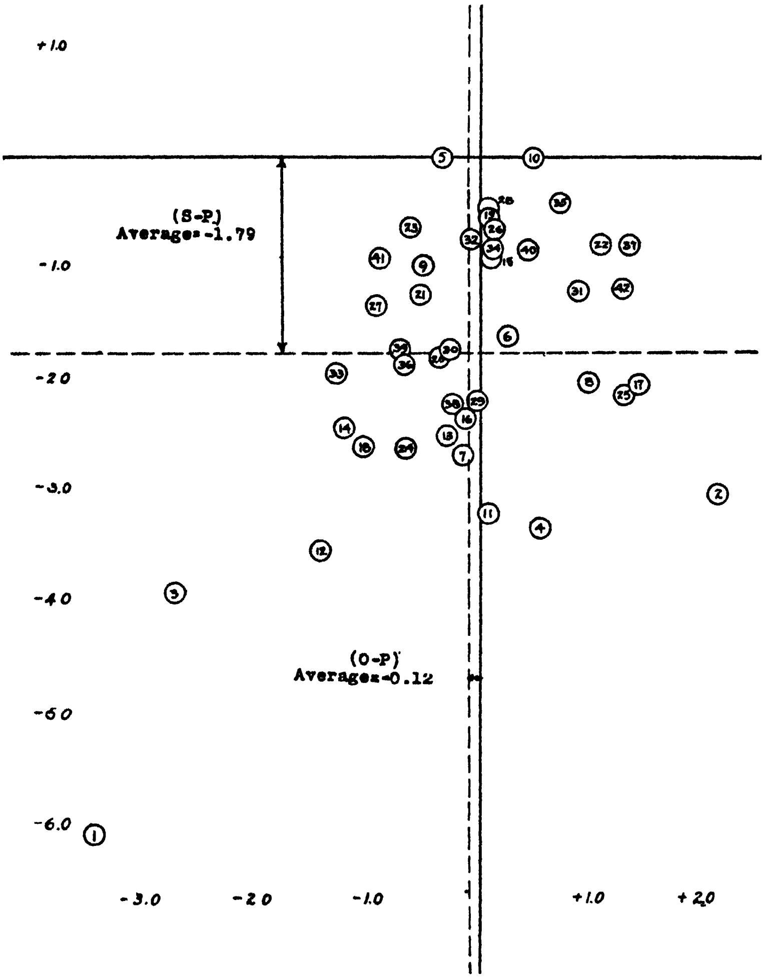


FIGURE 123.—Low line, including all matings. Correlation between degrees of selection (S—P) and departures of offspring from the means of their parental generations (O—P). For explanation see figure 122.

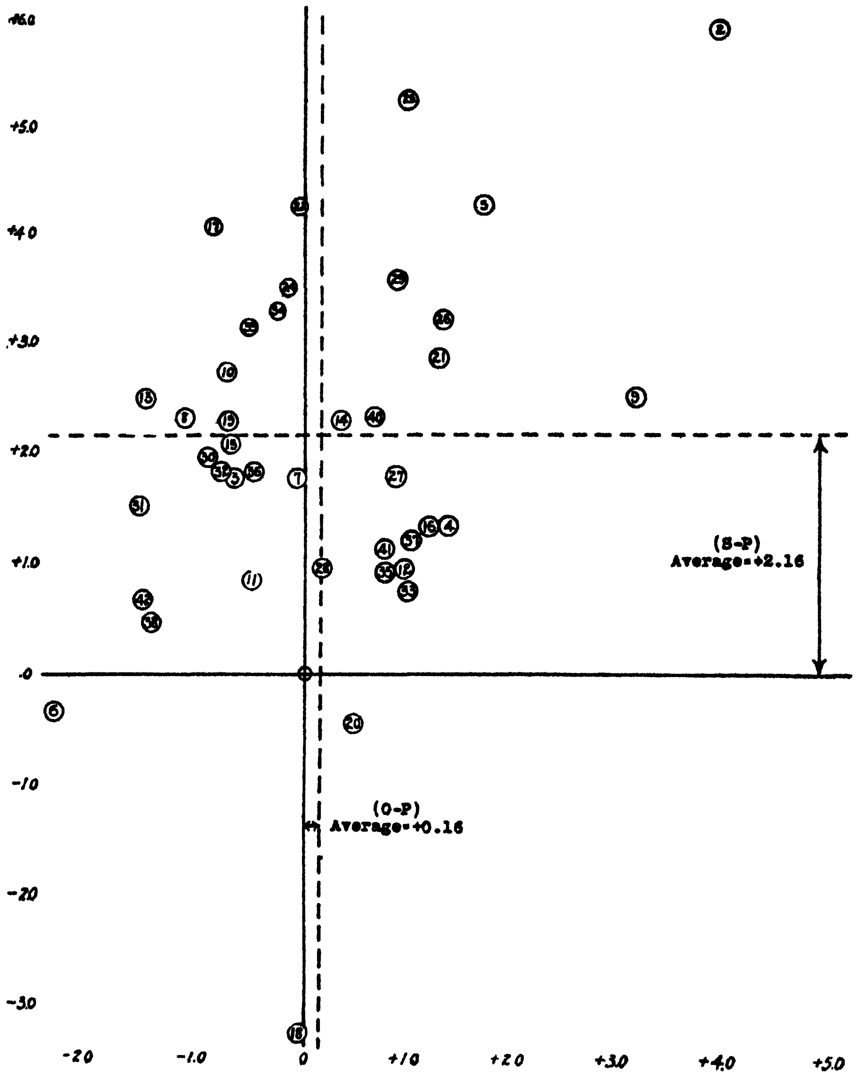


FIGURE 124.—High direct line. Correlation between degrees of selection (S—P) and departures of offspring from the means of their parental generations (O—P). For explanation see figure 122.

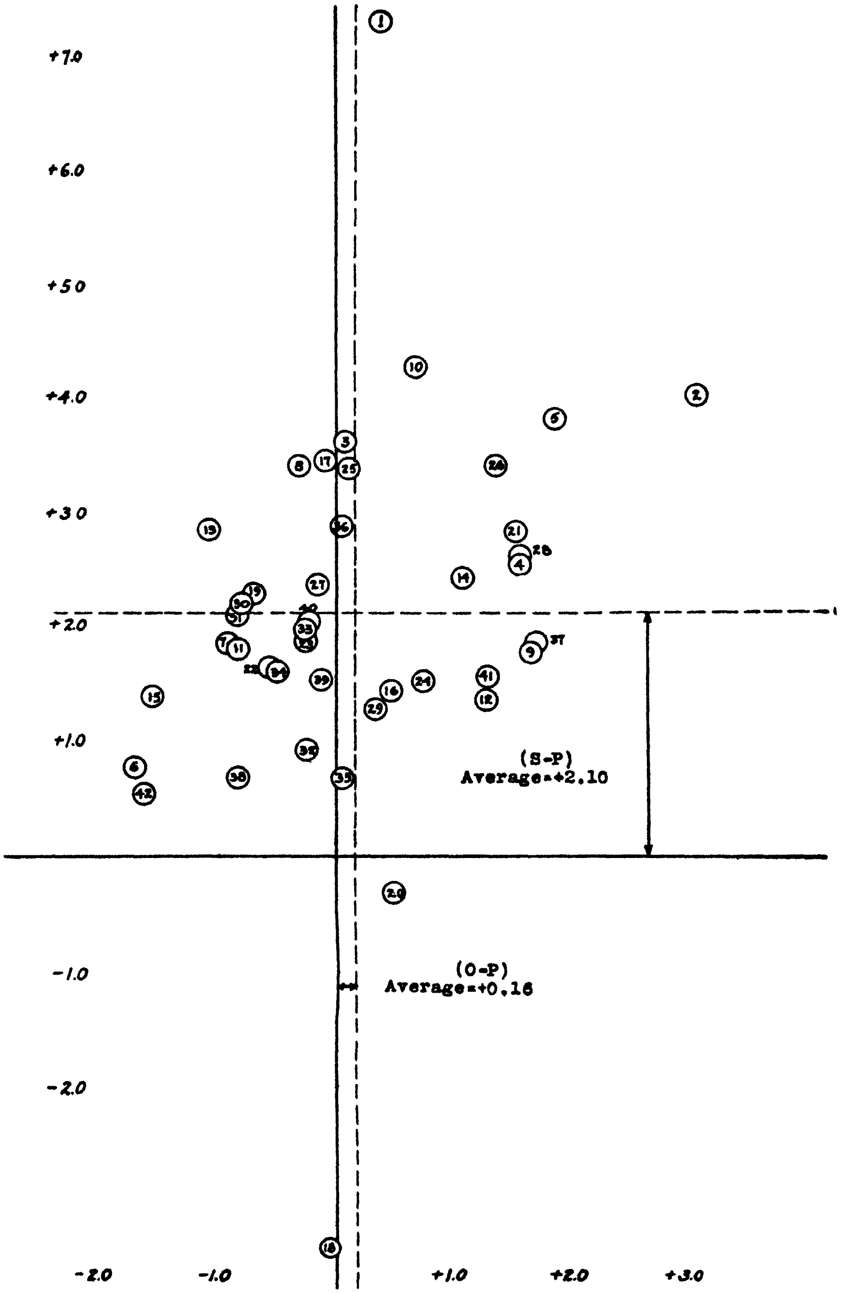


FIGURE 125.—High line, including all matings. Correlation between degrees of selection (S—P) and departures of offspring from the means of their parental generations (O—P). For explanation see figure 122.

The average degree of selection in the low line is −1.71 ten-percent factorial units, and the average effectiveness −0.12 or one-fourteenth of the degree (figure 122). Including all matings, the degree is −1.79 and the effectiveness again −0.12, one-fifteenth of the degree (figure 123). In the high direct line these values are +2.16 for degree and +0.16 or one-fourteenth of the degree for the effectiveness (figure 124). Including all matings, the values are +2.10 for the degree and +0.16 or one-thirteenth of the degree for the effectiveness (figure 125).

It is evident that the facet differences when taken throughout the experiment as a whole are largely not of hereditary value. An analysis of the effectiveness of the selection shows clearly that only a very few of the selections were markedly effective. For the great majority of the generations the low-facet parents in the low line and the high-facet parents in the high line gave indifferently lower or higher offspring than the means of the parental generations. The total effectiveness can be ascribed to a very few generations, principally 1 and 3 in the low line and 2 in the high line. This agrees with the conclusion reached previously by a study of the ranges, means and standard deviations, that only those selections which eliminated definite germinal factors were effective, and that the number of such differences is small.

CONCLUSION

In order to understand the nature of the selection effect, it is necessary first of all to consider the two most striking mutations of bar. In the upward direction there is a sudden change from bar to full eye in about one individual in 1600. In the downward direction there is a sudden change to ultra-bar with a frequency considerably less than that to full. If these mutations are considered in the experiments under discussion, upward selection effected a change to full eye in the very first generation and downward selection a change to ultra-bar in the second generation, though it took a generation or two longer to establish full and ultra-bar in homozygous condition.

If these two striking mutations are left out of the consideration, the results of the selection can be explained on the view that there is an early effective sorting of definite germinal differences which were present in the unselected population. This early sorting is completed in from one to five generations, and there is no further progress except at one point in the low line and at two points in the high line.

In the low line immediately following the 12th generation, there is a shift to a slightly lower level. This is undoubtedly a further sorting of original differences because the drop affects only the upper limit of the range, the lower limit remaining unchanged.

In the high line, the early effective period is followed by no further change until the 20th generation. After that time two independent events occur, each of which has its effects upon facet number. First, there is a marked upward shift in the females, the males remaining unaffected. This shift which persists for eight generations is explained by the appearance of a sex-linked lethal factor affecting facet number which kills all males possessing it but allows the heterozygous females to live. That a lethal factor is actually present is shown by the ratio of two females to one male in these generations. The drop after the 28th generation is to be explained by the fact that only half of the females in each generation contain the new lethal factor, and with the large fluctuation in facet number due to non-heritable factors, there is certain sooner or later to be a choice of a female with high facet number due to such non-heritable factors rather than to the heritable one. The lethal is thus immediately eliminated and the facet average of the females drops.

The second shift takes place following the 25th generation and is a sorting phenomenon similar to the one mentioned as occurring in the low line. The upper range of the males remains unchanged, but the lower range of both females and males shifts upward to a higher level as is to be expected on the hypothesis of the elimination of a germinal factor for low facet number which up to this time had been obscured by non-heritable fluctuations. The action of the upper limit of range of the females with respect to this change is obscured by the more pronounced temporary effect of the lethal factor discussed above.

These results are in agreement with the general trend of recent work on the nature of the selection effect. A critical discussion of this work is, however, reserved for another occasion. The existence of defintie hereditary factors on which selection can act by elimination of certain ones and preservation of others seems to be clearly demonstrated. New germinal differences arise by mutations whose origin, as shown elsewhere, is independent of selection. When they have arisen they are, however, subject to the further action of selection. In the present material the striking character of the mutations makes possible the early change to full eye by upward selection and to ultra-bar by downward selection.

SUMMARY

1. Selection for high and low facet number in a white bar race of Drosophila quickly gave full eye on the one hand and ultra-bar on the other, because of the appearance of definite mutations for these characters.

2. The origin of these mutations is independent of selection which has merely the function of preserving or eliminating them after they have appeared.

3. Neglecting these striking mutations, selection causes a rapid decrease in mean facet number in the low line during the first one to three generations. This decrease is accompanied by a striking decrease in variability. There is, however, no extension of the range downward, the whole change being brought about by the elimination of high-facet individuals.

4. From the fourth to the twelfth generations selection is without effect.

5. Between the 12th and 14th generations there is a further lowering of the mean with a decrease in variability. Again there is an elimination of high facet numbers but no downward extension of the range.

6. From the 14th to the 42nd generations there is no further effect of selection.

7. Neglecting the striking mutations as before, selection causes a rapid increase in mean facet number in the high line during the first five generations. This increase is accompanied by a striking decrease in variability. There is, however, no upward extension of range, the change being brought about by the elimination of individuals with low facet numbers.

8. From the 5th to the 20th generations, selection is without effect.

9. From the 21st to the 28th generations the means of the females are temporarily higher than before, but the males are unchanged. The sex ratios indicate that this increase is due to the appearance of a sex-linked lethal factor for increased facet number. At the 28th generation the lethal factor is lost and the facet number drops again.

10. At the 25th generation there is a rise in the means, apparent in the males but obscured for the moment in the females by the sex-linked lethal just mentioned. There is at the same time an elimination of individuals with low facet numbers but no upward extension of the range.

11. These facts and others discussed in the paper demonstrate the existence of definite hereditary factors for facet number which are sorted out by selection. The major ones are caught in the selection net in from one to five selection generations. Some of the minor ones are

protected by the non-heritable fluctuations until a later period when they also are separated out.

12. Apart from this sorting effect of pre-existing differences which must necessarily be completed sooner or later, the appearance of definite mutations causes new germinal differences which are acted upon by selection as they appear.

13. Of the mutations noted during the course of the selection, the striking one to full eye appeared four times in the high line and three times in the low line.

14. The mutations to ultra-bar appeared at least twice, once in the 2nd generation of the low line and once in the 22nd generation of the high line. Several other very low individuals that died without offspring were probably ultra-bar also.

15. A sex-linked lethal factor causing increase in facet number appeared in the 21st generation of the high line.

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THE INFLUENCE OF ALCOHOL ON THE FERTILITY OF WHITE RATS

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INTRODUCTION

This paper deals with the effect of alcohol upon the number and size of litters produced by white rats. Data upon the fertility of alcoholized rats and their descendants have been gathered from breeding incidental to a study of the influence of alcohol upon the behavior of these animals. The summaries presented are based upon 177 pairs of rats, which produced in all 1755 offspring. In all the experiments dealing with alcoholism this is apparently the first time that the fertility of treated animals has been tested by comparisons with full brother-and-sister controls. The results indicate that heavy as well as light doses of alcohol tend to reduce the sizes of the litters from treated rats and their descendants. Heavy doses reduce the number of litters produced by the treated rats and increase the number of litters produced by their descendants. The first result is interpreted as due to a modification of the germ-plasm, while the second is believed to support the hypothesis that the alcohol has raised the numbers of litters in the second generation through the elimination of the litters in the first generation that bore the less fertile germinal material.

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[1] These experiments have been carried out with the coöperation of E. M. VICARI.

METHODS

Material

Two series of experiments are presented. The rats in the first series came from colonies, believed to be unrelated, at different laboratories; the matings were made between different lines and only one litter per pair was bred. The second series of experiments was derived from three litters from the Wistar Standard Stock and from one mixed strain of rats from stock held over from the first series; all matings were made between full brothers and sisters. Children and grandchildren of the litters from WISTAR were used as the original treated animals.

In order to demonstrate the relationships of the animals used as original material, pedigree tables are given. The matings in the first series are shown to be between unrelated rats in figure 1. In strains A, B and C from the three Wistar litters in the second series (figures 2, 3 and 4) there is no complication to require comment. A fourfold origin is shown for strain LHKC (figure 5); this starts with four unrelated rats in two pairs which produced two pairs in which the fathers were brothers and the mothers were sisters. Attention is called to the fact that two pairs of ancestors in this strain were treated with alcohol; the dosage was slight and there is reason to believe that this has left slight racial influence. But even granting that it did leave its effect in the race, this would be equally distributed among the treated animals and their controls in the subsequent generations, so the final comparison of the treated and controlled rats would be unaffected. The pedigrees reveal a lack of uniformity among the different sets of treated and control pairs in regard to the amount of inbreeding. The numbers are too small to justify a detailed study of the different degrees of inbreeding. However effective this factor may be in the absolute results, the comparative results are no more disturbed by this than by the alcoholic history in strain LHKC.

Controls

Although adequate controls are a primary requirement of all experimental studies, in work along the present lines the suitability of the controls used has very often been open to question. Frequently the controls came "from the same dealer," or they were just normal or comparable, or were "guaranteed not to be brothers and sisters," or their source is not mentioned. What consitutes acceptable controls

MATINGS IN THE FIRST SERIES.

Father's Parents	Treated and Normal Pairs			Mother's Parents
5A/15L	N	79/62		21E/33F
	A	78/64	(256)	
21E/33F	N	59/76		5A/15L
	A	60/74	(232)	
F/9H	N	56/80		14J/2L
	A	52/82	(350)	
	N	54/81		
	A	51/84	(282)	
	N	55/86		
16L/9H	N	98/103		42K/15L
	A	95/102	(392)	
42K/18C	N	124/109		14J/13F
	A	128/111	(242)	
	N	124/112		
	A	128/110	(298)	
42K/18C	N	124/132		14J/9H
	A	128/134	(324)	
16L/9H	N	97/65		42K/18C
	A	101/69	(324)	
	N	93/67		
	A	95/68	(324)	
	N	97/70		
	A	101/71	(427)	
	N	98/127		
	A	95/123	(322)	
	N	97/129		
	A	101/125	(324)	

FIGURE 1.—Pedigrees of the treated pairs and their controls in the first series. All matings are between unrelated rats. The first number in each pair is the male. N stands for normal (controls): A stands for alcoholized (treated) pairs. The numbers in parentheses are the numbers of days of alcohol treatment (light dosage) given the females, plus the number of days given the males, that could affect the young. The litters indicate the different sources of the parents.

varies with the training of the investigator. Controls that may fully satisfy the demands of a psychologist or an anatomist may seem quite

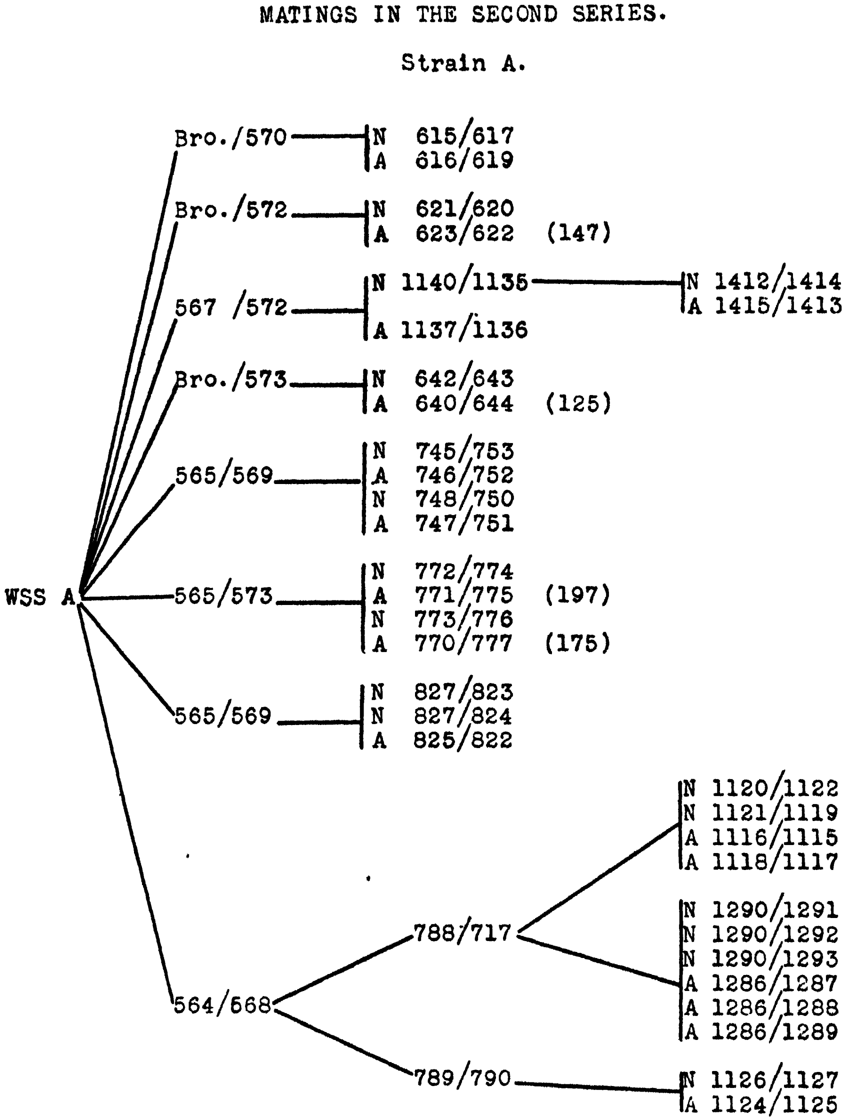

FIGURE 2.—Pedigrees of the original treated pairs and their controls in strain A of the second series. All matings are between brothers and sisters. The treated pairs (A) were given a maximum daily dose. The numbers in parentheses are the numbers of the days of alcohol treatment given the females, plus the number of days given the males, that could affect the offspring born in the first litter. Later litters were affected by increasingly longer treatment. Males were treated continuously; females were not treated while nursing a litter. No number in parentheses is given after a treated pair when no litter was produced. The same symbols are used as in figure 1.

worthless to a geneticist; for the geneticist is more keenly aware of the great variety of heritable differences, the presence of which may vitiate the results, and he demands the greatest possible similarity of germ-plasm between tests and controls. In the present work the brothers of the treated males and the sisters of the treated females were used as controls.

The division of each litter, when 28 days old, into those to be treated and those to be used as controls, was made on the basis of equal weights and equal numbers of both sexes. In questionable cases the advantage in weight was given to the group to be treated, so the average weights at 30 days show the treated rats a little higher than the controls. The matings are all of one type: between the original males and females or their descendants and between the original control males and females or their descendants. The matings between the controls parallel those between the experimental animals, so that each group of tests in each strain and each generation has its own particular, corresponding group of controls. The first series, as stated, deals only with the fertility of treated rats; the second series proceeds to the fertility of the grandchildren of treated rats. Since the later generations were produced by inbreeding, the closest possible relationship for the tests and controls in the successive generations was for them to come from the same single pair of grandparents or great-grandparents; they are thus raised at the the same time, after the same numbers of generations of inbreeding.

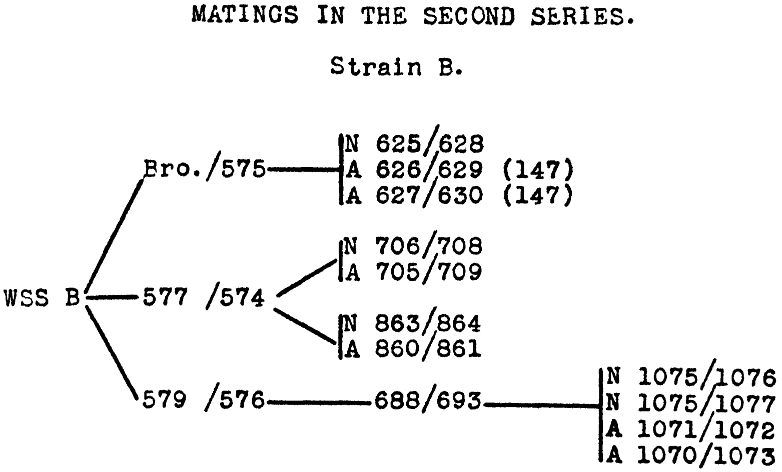

FIGURE 3.—Pedigrees of the original treated pairs and their controls in strain B of the second series; for details see legend of figure 2.

A few more than half the pairs reported are controls. The treated and control pairs from the same parents are called a set; in some cases a set included more than one treated and one control mating.

Treatment

The treatment of these rats was by means of the inhalation method now made familiar by the work of STOCKARD and of PEARL. For the present purposes it seems quite unnecessary to repeat the detailed account of the vapor tanks used in this process, as it has already been given elsewhere (MACDOWELL and VICARI 1921). In the first series the rats were placed in the tanks daily from the age of 28 days up to the birth of the young. Beginning with thirty minutes a day, treatment

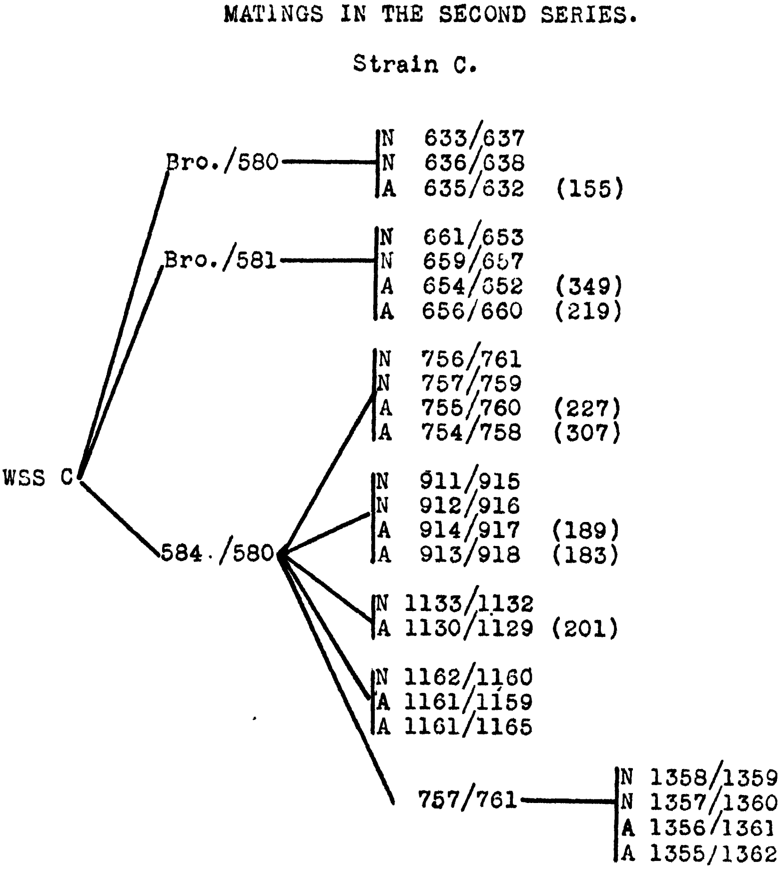

FIGURE 4.—Pedigrees of the original treated pairs and their controls in strain C of the second series; for details see legend of figure 2.

was gradually increased to a maximum of 90 minutes a day at the age of 50 days. There was great variability in the alcohol content of the air in the tanks, but the treatment was relatively light throughout, since in no case was the behavior of a rat noticeably modified when removed from a tank. In the second series, also beginning at 28 days, the rats were rapidly worked up to a maximum dosage that daily rendered them dead drunk, that is, completely anaesthetized and motionless. PEARL (1916, p. 679) speaks of the practical impossibility of inducing a typical drunken state of muscular incoördination in animals by the inhalation method, but his observations obviously did not cover rats, or, as STOCKARD (1918, p. 131) has since pointed out, guinea-pigs. The comparison of the

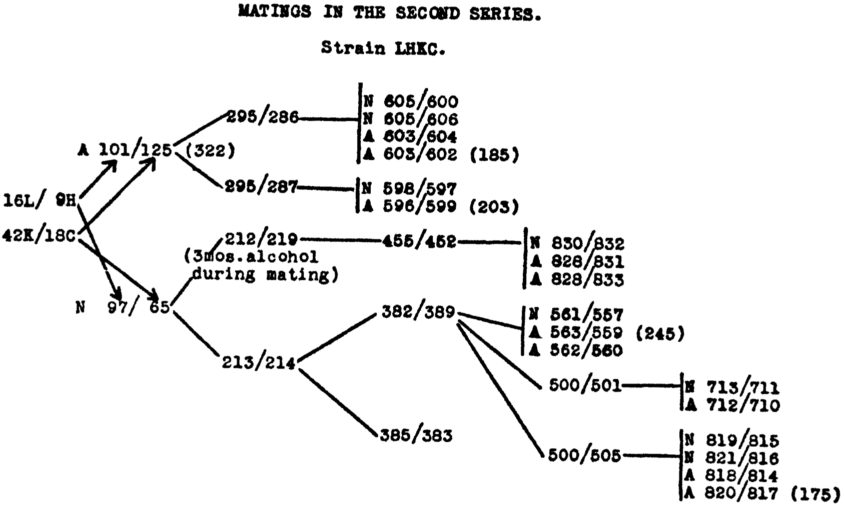

FIGURE 5.—Pedigrees of the original treated pairs and their controls in strain LHKC of the second series; for details see legend of figure 2.

two series will afford a chance for the different effects of relatively mild and maximum dosages to be manifest.

The number of treatments that could influence a litter is counted by adding the number of days the mother was treated up to the birth of the litter, to the number of days the father was treated up to conception, estimated at 21 days before the birth of the litter. In the first series with the light daily dosage, the average number per pair of effective treatments was 301.45; the range is between 232 and 427 treatments. In the second series, with the daily dose causing complete intoxication, the average number of treatments per pair that affected the first litters was 199.0; this average is unweighted by strains. The averages for each strain are: A, 161.0; B, 147.0; C, 228.4; LHKC, 202.0. The litters following the first were influenced by more treatments since the treatment of the fathers was continuous and the mothers were treated between the weaning of one litter and the birth of the next. The father was removed from the mother before the birth of a litter and returned when the litter was weaned.

Criteria

Since the present topic was merely a side line, the only criteria available to gauge the influence of the alcohol treatment upon fertility are: (1) the size of litters, and (2) the number of litters from the "test"[2] and control pairs during equal times. Since different pairs of rats were together for different lengths of time, owing to deaths, it is necessary to include only those records obtained while the pairs in one set of test and control matings were extant. In some cases a test mating was broken up without producing a litter before the control litter was born; following the rule, such a set is eliminated from the summaries. In the cases where a mating was broken up in sets including two test and two control pairs, the records of the other three pairs are given; but no record is given of pairs for which there was not one or more comparable pairs mated for an equal period.

RESULTS

Litter size

Treated rats from untreated parents

In the first series (table 1, first line), records are given for thirteen pairs of treated rats and thirteen pairs of controls. Each pair produced one litter, with the average litter size 8.30 for the treated rats and 9.38 for the controls; the difference (see table 2, first line) is a reduction of 11.5 percent from the controls. Calculated from the probable errors based on the standard deviations, this difference is 1.4 times its probable error.

Placed here on account of having received the same alcohol treatment as the first series, are six pairs of treated rats and seven pairs of controls raised by inbreeding from two litters received from the Wistar Institute. These rats have been known in the laboratory as the Wistar Defectives. They have not been mentioned before this and do not appear again. The matings were between brothers and sisters. Not a single litter was given by the treated pairs and the controls gave a litter apiece from four

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[2] The term "test" will be used to indicate experimental animals, both those directly treated as well as their descendants. Stockard uses the term "alcoholic" in this sense.

TABLE 1

Data on the fertility of white rats. Numbers of pairs, litters, and rats, in the different generations of the experiments, subdivided and combined in various ways. The averages for litter size and number of litters per pair are given. For further comparisons see tables 2 and 3. The subdivisions of strain LHKC are based upon the different inbred generations to which the treated animals belonged. The signs "ch," "G. ch" and "GG. ch" stand for children, grandchildren and greatgrandchildren, but they have no other significance than indicating successive generations.

	CONTROLS					TESTS				
	Pairs	Litters	Rats	Rats per litter	Litters per pair	Pairs	Litters	Rats	Rats per litter	Litter per pair
Treated from untreated										
First series	13	13	122	9.38	1.00	13	13	108	8.30	1.00
Second series: Strain A	17	29	179	6.17	1.05	16	5	25	5.00	0.31
Strain B	5	6	32	5 33	1.20	6	2	13	6.50	0.33
Strain C	12	29	225	7.75	2.41	12	17	97	5.70	1.41
Strains A, B and C	34	64	436	6 81	1 88	34	24	135	5.62	0.70
Strain LHKC"ch"	3	9	67	7 44	3.00	3	3	26	8.66	1.00
Strain LHKC"G.ch"	2	3	19	6 33	1 50	4	1	1	1 00	0 25
Strain LHKC"GG.ch"	3	11	61	5.54	3 66	3	4	31	7.75	1.33
All strain LHKC	8	23	147	6.39	2 87	10	8	58	7.25	0.80
All strains										
First litters	42	36	247	6 86	0.85	44	18	111	6.16	0 40
All litters	42	87	583	6 70	2 07	44	32	193	6 03	0 72
Treated from treated										
Second series: Strain C	7	19	139	7.31	2.71	5	8	53	6 62	1 60
Strain LHKC	5	10	52	5.20	2.00	4	6	30	5.00	1.50
Strains C and LHKC	12	29	191	6.58	2 41	9	14	83	5 92	1.55
Summary of treated rats	54	116	774	6 67	2.14	53	46	276	6.00	0.86
Untreated from treated										
Second series: Strain A	3	6	34	5.66	2.00	3	9	53	5.88	3.00
Strain B	2	3	20	6.66	1 50	2	1	2	2.00	0.50
Strain C	2	1	7	7.00	0.50	2	2	14	7.00	1.00
Strains A, B and C	7	10	61	6.10	1.43	7	12	69	5.75	1.71
Strain LHKC	3	5	32	6.40	1.33	3	8	41	5.12	2.66
All strains	10	15	93	6 20	1.50	10	20	110	5.50	2.00
Untreated from untreated from treated										
Second series: Strain A	3	3	24	8.00	1.00	1	0	0	0	0
Strain C	2	7	39	5.57	3.50	2	6	53	8 83	3.00
Strain LHKC	8	9	64	7.11	1.12	8	19	92	4.84	2.37
All strains	13	19	127	6.68	1.46	11	25	145	5.80	2.27
Summary of untreated rats	23	34	220	6.47	1.47	21	45	255	5.66	2.14

TABLE 2

Litter size. Differences between the litter sizes of tests and controls in various generations of the rats compared with such differences in guinea-pigs. The figures for the guinea-pigs are calculated from the data of STOCKARD *and* PAPANICOLAOU *1918, tables 2 and 5.*

TREATMENT OR GROUP OF ANIMALS	WHITE RATS					STOCKARD'S GUINEA-PIGS				
	LITTERS		Differences — = tests less	Diff. / P E	% Differences Controls 100%	Litters		Differences — = tests less	Diff. / P. E.	% Differences Controls 100%
	T	C				T	C			
First series										
Treated from untreated...	13	13	−1.08±0.78	1.4	11.5	74	88	−0.26±0.09	2.8	9.4
Second series										
(1) Treated from untreated										
All litters	32	87	−0.67±0.34	1.9	10.0					
1st litters.............	18	36	−0.70±0.41	1.7	10.2					
"Balanced litters".......	13	13	−1.50±0.56	2.6	20.0					
(2) Treated from treated parents	29	14	−0.66±0.52	1.2	10.3	22	88	−0.23±0.11	2.1	8.3
(3) Untreated from treated parents..............	20	15	−0.70±0.48	1.4	11.2	109	88	−0.38±0.08	4.7	13.7
(4) Untreated from untreated parents and treated grandparents...	25	19	−0.88±0.56	1.6	13.1	43	88	−0.17±0.12	1.4	6.1
(1) and (2)..	46	116	−0.67±0.28	2.4	10.0	96	88	−0.26±0.08	3.2	9.4
(3) and (4)..............	45	34	−0.81±0.38	2.1	12.5	152	88	−0.32±0.07	4.5	11.5
All generations...........	91	150	−0.79±0.22	3.6	11.9	248	88	−0.30±0.07	4.3	10.8

The rats and guinea-pigs may be compared directly by the percentage differences and by the significances of the differences; when the difference is as much as three times the probable error it may be considered fully significant statistically. The differences for the guinea-pigs are statistically significant in more cases than for the rats; this is due to the smaller numbers of rat litters, since the combinations of generations show increased significance. The percentage differences for the rats and guinea-pigs are of the same order of magnitude but the rat differences tend to be somewhat larger. The heading "Balanced litters" refers to the averages, weighed by sets, excluding controls whose corresponding tests were not born. The 88 guinea-pig litters shown as controls in each generation are the same animals in every case.

out of the seven pairs. These control litters averaged 5.25 rats, which is 4.13 rats per litter smaller than the average of the out-bred control in the first series proper. This difference in the size of the control litters is probably due in part to the out-breeding in one case and inbreeding in the other. However, when Wistar Defectives are compared with the other three litters received from the WISTAR INSTITUTE (strains A, B and C described below; see table 1, fifth line), the necessity for strict genetic control in studies of this kind is made obvious. Beneficial effects of alcohol could easily have been shown had these rats been used for controls and one of the other Wistar litters for treatment.

In the second series the breeding was continued in most cases beyond the first litter. Considering the strains separately, the treated rats in strains A and C give smaller litter averages than the controls, the differences being 1.17 and 2.05, respectively. In strains B and LHKC the treated rats give larger litters than the controls, the differences being 1.17 and 0.86, respectively. The rats used in strain LHKC came from three different generations of inbreeding. If the litters from this strain are divided into groups on the basis of the generation of their parents, two of the three group averages favor the tests. Taking all the strains in the second series together, the reduction in the size of the test litters is 10.0 percent of the controls; the actual difference is 0.67 ± 0.38, which is 1.9 times its probable error (table 2, line 2).

In order to give summaries that are possibly more strictly comparable with the first series, the averages of the first litters in all strains are given. There were 36 litters from the control rats with an average of 6.86 rats per litter. This makes a reduction in the size of the test litters that is 10.2 percent of the controls, against 11.5 percent in the first series; the actual difference in the second series is 1.7 times its probable error.

The most accurately controlled results of the size of first litters are obtained by counting only those sets of matings in which test litters were actually produced. This eliminates 17 control litters from the second series. A still further refinement in the comparison is obtained by giving each set of matings equal weight; the averages for the litters in sets containing more than one treated and one control pair are used, instead of the individual litter sizes. The average litter size for the test and control matings in each set is shown in figure 6. Each bar represents the test and control averages from one set of matings, that is, the first litters from the treated and control pairs that came from the same pair of parents. If the control average is greater than the test average, the difference is represented by a solid black portion of the bar: if the test average is greater, the difference is represented by broken lines. The order of bars is arbitrary; those favoring the tests are put together at the right. The test litters are smaller in five out of the eight sets of matings in the first series; in the second series, the test litters are smaller in ten out of thirteen sets. The final averages obtained by this method are as follows: first series, controls, 9.39 rats per litter, tests, 8.25, making a difference of 9.3 percent as against 11.5 percent by the first method; the second series, controls, 7.50, tests 6.00, making a 20 percent reduction (see table 2, "balanced litters"). the difference in the second series is 2.6 times its probable error. It may be noted that in spite of the small numbers this is the only difference in litter size in a single generation that approaches three times its probable error.

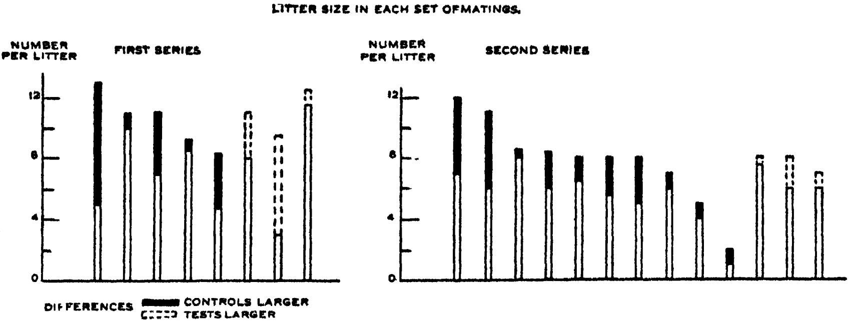

FIGURE 6.—Average litter size in each set of test and control matings in the first and second series. Each bar represents the test and control averages for one set, based on first litters only. When the control average is greater than the test average the difference is shown by a solid portion of the bar; when the test average is greater, the difference is shown by broken lines. The sets in which the test averages are greater have been arbitrarily placed at the right.

This balancing of test and control litters makes but slight difference in the first series since it does not involve the omission of any litters, but in the second series it raises the average of the controls so that the percent reduction of the test litters is nearly doubled. Clearly the control litters unbalanced by test litters must have been smaller than the controls whose corresponding test matings were successful. The average of the 17 control litters not included above is 6.17 rats per litter. So there appears to be a common genetic basis for the absence of test litters and the small size of control litters. If this low control average alone were compared with the primary average of the 18 test litters (6.16) one would conclude that the alcohol treatment had no effect upon litter size. This conclusion would be incorrect, since these particular normals, although from the same strains and blood lines, are really not controls for these particular tests; they differ genetically as well as experimentally. The sorting out of this group was done consciously in this case, but in cases where the controls come merely from the same dealers or the same blood lines, the same sorting out may be done entirely unconsciously and lead to spurious conclusions.

With the exception of the test litters in two lines when the strains are taken separately, all the averages tend to indicate a general reduction in the size of the litters from treated rats as compared with litters from their untreated brothers and sisters. This appears in the first series where the alcohol dosage was light and the matings out-crosses; it appears in the second series where the alcohol dosage was maximum and the matings between brothers and sisters. It appears when first litters are taken alone and when all litters are included. The difference is about 10 percent of the controls in most cases; but in no case is any difference by itself as great as three times its probable error. However, in the most closely controlled averages, that is, excluding sets in which the test litters were not produced, the difference in the size of first litters closely approaches three times its probable error (2.6 times).

Treated rats from treated parents

A random selection of rats in the second series was itself subjected to alcohol fumes. Records are given of the results of nine matings between these rats and twelve matings between their own particular controls. In this generation the controls can obviously not be brothers and sisters, but they hold the closest relationship possible, namely double first cousins from the same single pair of grandparents. Considering all litters born in equal periods, the tests produced litters on the average 10.3 percent smaller than the control litters; the difference (0.66 ± 0.52) is scarcely larger than its probable error. The only strains that happened to be included in this generation are C and LHKC; taken separately, each gives a test average smaller than the control. Treating grandparents as well as parents does not increase the reduction in the size of the litters produced; when parents alone were treated the difference between test and control averages for all litters was 10.0 percent; when the grandparents also were treated, the difference was 10.3 percent.

Untreated rats from treated parents

Ten pairs from the original treated rats are compared with ten pairs of their particular controls. Strain A gives a higher average for the test litters; strain C gives equality; strains B and LHKC give lower averages for the test litters than for the controls. The averages of all the rats together in this generation favor the controls to about the same degree as in the preceding generations; the difference (0.70 ± 0.48) is 11.2 percent of the control average and 1.4 times its probable error.

Untreated rats from untreated parents and treated grandparents

The fertility was tested of 11 pairs of test rats and 13 pairs of their immediate controls, produced in the preceding generation; that is, untreated rats from untreated parents and treated grandparents. The one test pair in strain A gave no litter; strain B was not represented; strain C gave the average of the test litters considerably above that of the controls (difference 3.24 rats) and strain LHKC gave litters that average smaller than the controls (difference 2.27 rats). Putting all strains together the difference is in favor of the controls (13.1 percent of the control average); it is 1.6 times its probable error.

Summary on litter size

In the generations following the first, with and without further alcohol treatment, there appears a reduction in the size of test litters. When the totals are considered, this difference throughout is of about the same percent of the respective controls. When the pairs are separated according to the strains, considerable irregularity in the comparisons is revealed; but this separation leaves very small numbers behind each average so the irregularity is not surprising, especially when the difference of the totals is as small as 10.0 percent. Statistically, none of the differences in the totals is great enough to be considered significant. The absence of statistical significance may be due either to the small size of the sample, or to the absence of any real difference however large the samples might be made. Taken singly, the generations from rats directly treated show no difference in litter size from generations further removed from treatment. The combination of the two generations in which the rats were themselves treated may be compared with the combination of the two generations in which the rats themselves were not treated. When the generations are so combined, the differences in favor of the controls are 0.67 and 0.81; their significance is raised to 2.4 and 2.1 times their respective probable errors. Combinations of generations give the same results as single generations,—similar reduction in litter size whether the rats themselves are treated or only their parents or grandparents. This result justifies adding all the generations together to get larger numbers. When this is done the difference becomes 3.6 times its probable error and is therefore fully significant. As the number of litters is increased the statistical significance of the result is raised, so that there is some basis for the belief that larger numbers in each generation would show significant differences.

Number of litters

As stated above, the numbers of litters given in the summaries include only those produced in equal times by test pairs and their particular controls; some sets cover longer times than others, but the opportunities for the tests and controls to produce litters are equal, whether individual sets or whole generations are considered. The number of litters per pair differs from the number of rats per litter in that it is relative and has no significance if taken away from the directly comparable figures. The data are given in table 1. Since only one litter per pair was raised in the first series the discussion is limited to the second series. Comparing the numbers of litters per pair from the treated rats and their controls, every subdivision of the table shows the treated rats producing fewer litters than the controls. In order to obtain a measure of the significance of this difference the probable error is calculated upon the percent reduction in the number of test litters in this whole generation. The controls produced 2.07 litters per pair; 44 treated pairs at this rate would have produced 91.08 litters, whereas they actually produced 32 litters; 59.08 expected litters (64.86 ± 3.37 percent) did not appear (see table 3). The

TABLE 3

Number of litters. Differences between the number of litters produced by test rats and guinea-pigs and the number of litters normally expected. Minus signs indicate that the percents are below normal expectation; plus signs indicate that the percents are above normal expectation. The data for the rats are based upon the number of litters produced by the test and control pairs in equal periods. The figures for the guinea-pigs are derived from data given by STOCKARD *and* PAPANICOLAOU *1918, p. 285; they are based on the numbers of failures of matings among the tests and controls. A male and female together during a period of heat was counted as a mating. In all cases these differences may be considered statistically significant.*

GENERATION	WHITE RATS		STOCKARD'S GUINEA PIGS	
	% difference between No litters produced and No. normally expected	$\frac{\text{Diff}}{\text{P. E.}}$	% difference between No litters produced and No. normally expected	$\frac{\text{Diff.}}{\text{P. E.}}$
Second series				
Treated from untreated	−64.86 ± 3.37	19.2	− 8.40 ± 2.87	2.9
Treated from treated	−35.45 ± 6.91	5.1	−19.59 ± 5.28	3.7
Untreated from treated	+33.33 ± 8.29	4.0	− 8.26 ± 2.50	3.3
Untreated from untreated from treated	+55.60 ± 8.40	6.6	−11.32 ± 3.56	3.2

percentage difference, being 19.2 times its probable error, is significant beyond all question.

Since comparable treated and control matings were always made at the same time, the number of days that the males and females were together before the birth of the first litter gives some further light upon this reduction in the number of litters. Figure 7 represents the differences, in days, between the first test and control litters in each of the sets of matings included above in figure 6, that is, those sets in which the test litters were born. The horizontal line indicates equality; distances above show the number of days after the birth of the control litter before the tests were born, while distances below the line show the number of days the tests were born ahead of the controls. The situation in each set is shown by vertical lines; starting with those in which the test litters were longest delayed, the sets are given in the order of decreasing differences. In the first series the tests were born later in two sets, earlier in two sets and on the same day in four sets. The two sets in which the controls were slower in appearing, show smaller differences than the two sets in which the tests were slower, so the final averages favor the controls. The second series gives a very different story. In nine sets the tests were born from 2 to 48 days after the controls; in three sets both were born on the same day; in one set the tests were born 23 days ahead of the controls. So there is a tendency in the second series for the test litters to be slower than the controls in appearing; in the first series such a tendency is practically absent. Apparently this difference between the two series can be credited to the difference in the amounts of alcohol given; with small doses the birth of a litter is little delayed, but with large doses there is a strong tendency to delay. This result is suggestive in connection with the fact that all the expected litters were produced in the first series, while there was a great reduction in the number of test litters in the second series.

Turning to the numbers of litters produced in the later generations, the treated rats from treated parents also produced fewer litters than did their controls. Each strain separately shows this. Considering the whole generation, 14 litters were produced out of a possible 21.69, judging from production of the controls; that is, 7.69 (35.45 ± 6.91 percent) litters are missing. The percentage reduction is 5.1 times its probable error and is accordingly to be considered statistically significant.

Coming to the rats not directly treated (table 3, lines 3 and 4), untreated from treated parents produced 38.3 ± 8.29 percent more litters than the controls, and the untreated rats from untreated parents and treated grandparents produced 55.6 ± 8.4 percent more than the controls. Both of these differences are fully significant.

Summary of numbers of litters

The number of litters is strongly reduced and the time of birth delayed, when the rats themselves are treated, but when the treatment is limited to the parents or grandparents of the rats, the number of litters is increased, so that the test animals produce significantly more litters than the controls. These are very different results from those on litter size which show that litters from test rats seem to be slightly smaller than from the controls whether the rats were treated themselves or came from treated ancestors.

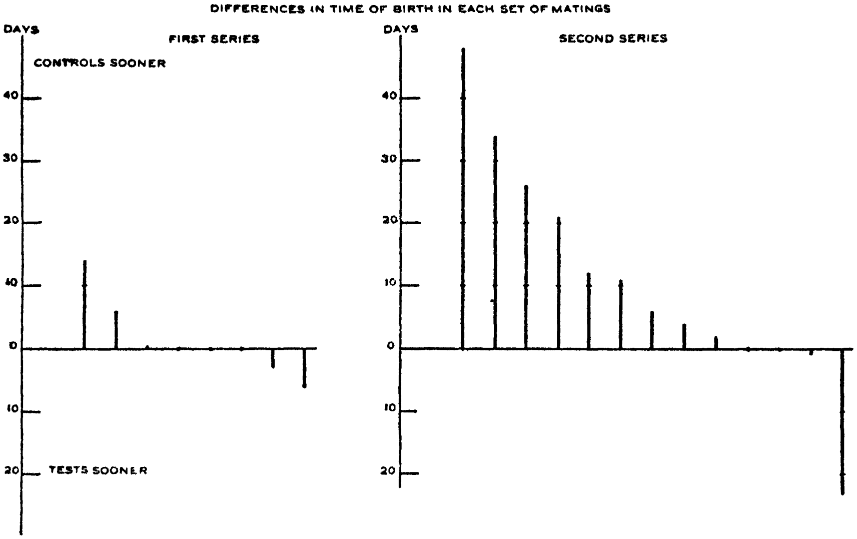

FIGURE 7 —Differences in days between the birth of the first test and control litters, in each of the sets of test and control matings in which test litters were born. Distances above the horizontal line indicate the numbers of days the controls were born ahead of the tests; distances below the line show days that the tests were born ahead of the controls. Points on the line indicate sets in which the test and control litters were born on the same day.

It should be noted that this reduction in the number of litters in the treated generation and the increase in the later generations is by far the most positive and unquestionable result here reported. Some doubt may linger as to the very striking uniformity in the percent reduction in the size of test litters in various generations and groupings and under different systems of breeding and different intensities of treatment. On the other hand, the precise coincidence of the immediate presence of large doses of alcohol and the strong reduction in the number of litters followed by a relative increase in the number of litters in later generations significantly above the controls, is supported by incontestable evidence. In searching for the meaning of this result, the obvious interpretation is that of PEARL (1917), namely, that the alcohol has acted as a selective agent. PEARL applied this principle to his results from the alcoholic treatment of fowl by supposing a direct elimination of the more inferior germ-cells. But in this case, it is not poor germ-cells that are eliminated; it is whole litters that are prevented from appearing through some reaction of the parents. There is a germinal basis for this reaction; possibly it is connected with a natural low reproductive capacity. The litters that are produced by treated rats carry germinal material that has demonstrated its ability to produce litters in the presence of alcohol; so rats from these litters in turn produce more litters, even in the presence of alcohol, than did the preceding generation as a whole. This is shown by the fact that the reduction in the number of litters produced by treated rats from treated parents was 35.4 percent, whereas the preceding generation showed a reduction of 64.8 percent. As soon as the alcohol is not immediately present (children of treated rats) the tests are found to bear germ-plasm that is more fertile, as measured by the numbers of litters, than the controls; for the controls still bear all grades of fertility while the tests lack the lowest grades.

Fertility, in mammals especially, is influenced by parental somatic conditions as well as by the constitution of the eggs and sperm; it is upon such parental conditions that the alcohol appears to have worked in reducing the numbers of litters, and not upon the germ-cells themselves. On the other hand, the constant reduction of litter size, not being interpretable as due to selective elimination, appears to be a heritable modification of the germinal material.

DISCUSSION

Although no general summary of the literature will be presented, certain of the more notable investigations will be cited for purposes of comparison. The closest comparison available is with the experiments of STOCKARD and PAPANICOLAOU; the following discussion is confined to their most recent publication (1918) on the effect of alcohol on guinea-pigs. This paper contains the most reliable data on guinea-pigs that have been presented. In tables 2 and 3 are given comparable averages, percents and probable errors, calculated from data in the above paper. The guinea-pigs were out-bred and took in new blood in each generation;

the controls are not parallel, pair for pair, with the experimental animals, although they did come from the same blood lines and some of the same individuals appear at different times as controls and as tests. In spite of these differences from the rat experiments, there appears a striking similarity in the percent reduction in the size of test litters. There is more variability in the percents from the guinea-pigs, but this may very possibly be connected with the fact that one control average has served for all generations, while for the rats each test average is compared with its own control of the same age, in the same generation, and in the same immediate environment. The larger number of guinea-pig litters raises the significance of the differences in litter size in the first two generations; the differences are respectively 2.8 and 4.7 times their probable errors. In the third generation the numbers and the significance are both much reduced. Agreeing with the rats, the immediate presence of alcohol seems to have no greater influence on litter size than the treatment of some ancestor, so the generations may be taken together without concealing any result; the difference between the test litters in all generations and the controls is 4.1 times its probable error.

Since the same control guinea-pigs were frequently paired with both experimental and normal mates, it was possible to make a summary in which the chances for genetic variability between the alcoholic and normal matings were reduced by one-half. But even so, half the chances for this variability remain. The results of mating normal males and females successively to normal and treated mates are shown in STOCKARD and PAPANICOLAOU 1918, table 9, p. 205. Using one common parent does not remove the chances for genetic differences between the two parents that are being compared on the basis of different experimental treatment. Not till these chances also are removed can there be offered "a most perfect control" that will provide "the crucial demonstration of the effects of alcoholism on the offspring" (loc. cit., p. 205). Rather than giving the most reliable comparison, this table seems actually less reliable than those that include whole generations and the whole mass of controls. The numbers are much reduced, while the heterogeneity of environmental factors is apparently increased by the data being taken from various generations. While the records and the entire table do appear to be perfectly consistent and very clear-cut as noted (loc. cit., p. 207), no mention is made of litter size; but the number of animals is given and the number of litters can be obtained by subtracting the number of failures from the total number of matings, so litter sizes can be determined. The litter size of the treated females shows a reduction of 18.9 percent of their controls; the treated males show an *increase* in litter size over their controls that is 16.8 percent greater than the controls. This result makes an inconsistency in the table and it does not agree with the extensive data in earlier tables indicating that the treated males tend to produce smaller litters than the treated females.

Although obtained in a somewhat different way, data are given by STOCKARD and PAPANICOLAOU (1918) that may be compared with the numbers of litters produced by the rats. For each experimental group and the control group they give the number of unsuccessful matings based on the oestrous cycle. The percents quoted in table 3 are the percent failures in the different test series less the percent of failures in the control series, consequently they show the percent difference between the number of litters produced and those normally expected. In all cases, whether alcohol is given directly to the animals themselves or only to the parents or grandparents, there are fewer litters from test animals. Moreover, the percent reduction in numbers of litters in all cases is of the same order of magnitude as the reduction in litter size. In spite of the similarity between the rats and the guinea-pigs in regard to litter size, there appears a marked difference when the number of litters is considered.

In studying the effects of chemicals upon animals, there has been a tendency to consider that general effects, such as decreased vigor, are shown in various ways, the different criteria being closely correlated; in such studies fertility has been regarded as a unit. But sterility is no longer regarded as unified in its causes as it is in effect, and the results with the rats indicate, provided there is no error in method or evaluation, that the number and size of litters are differently affected by alcohol; with the same assumption, the data on guinea-pigs indicate that these two criteria show similar effects of the alcohol treatment. In considering this difference in results from animals in the same order, it is important to note that the rats received larger doses of alcohol than did the guinea-pigs; they were brought to a state of complete anaesthesia, while the guinea-pigs were only partly intoxicated. Beside differing in the dosage, there is evidence that the rats naturally differ from the guinea-pigs in other ways as well as in the influence of alcohol upon the numbers of their litters. Rats can recover from complete alcoholic stupor day after day indefinitely, whereas STOCKARD states (personally) that a guinea-pig would not survive this treatment. No abnormalities, such as appeared in the guinea-pigs, have been produced in the rats in any generation by alcoholization.

In this connection there is a possibility that a certain difference in the data used in comparing the number of litters in the guinea-pig and rat experiments may give a more probable clue to the difference in results. The guinea-pig percents, as stated, are based on the numbers of matings in the sense of periods of heat; the rat percents, upon the litters produced in equal times by tests and controls. This leaves the chance for more factors to be operative upon the rat results than in the case of the guinea-pigs. Notably the rate of the appearance of heats can have no effect upon the data as given for the guinea-pigs, whereas it does play a part in the data given for the rats. It seems possible that the period between heats (the oestrous cycle) may be lengthened by alcohol treatment, and this may amount to suppression in animals with low reproductive powers as expressed perhaps by a tendency for a longer cycle. In this way the numbers of litters in the first generation would be reduced; in the untreated generations, through the elimination of litters carrying tendencies for longer cycles, the numbe of litters would be increased above the normal. That the heavily treated rats tend to require more time to produce a litter falls in line with this sort of an explanation.

Although chickens do not provide the same criteria of fertility as do mammals, it will be well to compare the results of PEARL'S (1917) work. He presents fertility data for fowls treated by inhalation with ethyl alcohol, methyl alcohol and ether. To make the closest possible comparison with the rats, only the matings involving ethyl alcohol will be cited. Eight Barred Plymouth Rock hens, four of them treated with fumes of 95-percent commercial (ethyl) alcohol, and four untreated (three of these, sisters respectively of the three treated birds, while the origin of the fourth is not shown in any of the tables), were crossed with a treated Black Hamburg cock. The untreated *half*-brother of the treated cock was relied upon to give the control results, but this control cock proved to be an unsuccessful breeder. So, instead of brothers and sisters of the treated birds, there was used a random sample of normal matings as controls. "For comparison with the results of the alcohol matings there will be used in this section of the paper the results from a random sample, comprising 22 matings, from all of the 1915 matings of normal untreated birds in which the female partner was a pure Barred Plymouth Rock." (PEARL 1917, p. 243.)

In regard to the number of eggs laid by the two sets of females there is certainly no indication of any reduction on the part of the treated birds; the available records of pullets (three treated and three controls) show that, up to June, the treated birds laid 311 eggs and the untreated birds laid 272 eggs. When the birds in all the experiments, including the three chemicals used, were averaged together, there appeared to be no difference at all between the number of eggs produced by those treated and those not treated. Of the eggs set from the hens treated with ethyl alcohol 46.1 percent were fertile and 72.9 percent of the fertile ones hatched, while 79.1 percent of the eggs set from the untreated hens were fertile and 58.0 percent of the fertile ones hatched. The percent fertile found among the random sample of untreated Barred Plymouth Rock females was 76.7 percent, and 57.8 percent of the fertile ones hatched. Treated females by treated males gave fewer fertile eggs than the untreated matings including Barred Plymouth Rock females that were used as controls, but the fertile ones have a higher hatching percentage. Compared with the same controls, the treatment of the male alone shows no modification in the fertility or hatching quality of the eggs from matings with untreated females. In the following season (PEARL 1916), after a whole year more of treatment, much the same results are found; there are fewer fertile eggs laid, especially when the dam is treated, and more of the fertile eggs hatch than in the controls.

These results, together with those from the otherwise treated birds, are believed to fall in line with the hypothesis that "alcohol and similar substances act as selective agents upon the germ-cells of treated animals" (PEARL 1917, p. 296). The eggs that did not develop are supposed to be those that in untreated animals would either have failed to hatch or become the more inferior individuals in the next generation. But for its plausibility, this theory could not stand firmly. For certainly there are ample grounds for objecting to the use of random samples of normal matings as controls; this is the old familiar criticism, so well stated in PEARL's own paper (1917, p. 139). Furthermore it is carefully explained (PEARL 1917, p. 242) that either partner in a mating may have an influence upon the hatching power of the eggs through an upset in the general metabolic balance. It is obviously unnecessary to make the same statement in regard to genetic influences, yet nothing is said about the males employed in these untreated matings and even their breeds are not revealed. As to the controls used in the 1916 matings, one is left to wonder whether the descendants of the random sample of normal matings used as the 1915 controls are employed, or whether some other entirely adequate control that happened to be at hand. The comparisons with this sort of control may very well have some meaning, as have other experiments with alcohol employing similar unsatisfactory controls, but they do not have the unusual significance naturally accorded them by the great number of readers who habitually confine their attention to the introductions and conclusions of papers. For it would not occur to such readers to question the item singled out in the summary of the first section, under number 2, as one of the chief points of methodology (PEARL 1917, p. 162), "Full brothers and sisters of treated [individuals] are used as controls," although further study of the paper would reveal this to be an inexplicable misstatement.

However far from ideal are the controls previously employed, one point appears to be well established; upon this the experiments with fowl, guinea-pigs and rats agree, namely, the alcohol treatment reduces the number of young produced. Since the number of young is the primary criterion of fertility, the immediate general effect of alcohol may be reduction of fertility. But unanswered by this finding is the outstanding problem of inheritance: is this reduction in output due to a modification that is inherited, or does the alcohol act exclusively as a selective agent? PEARL finds that neither a general deleterious effect, nor a specific germinal modification has been brought about in his experiments. He accounts for his findings on the basis of selective elimination alone. STOCKARD'S results, on the other hand, appear to show that in guinea-pigs some phenomenon of inheritance is involved. However, he takes up PEARL'S theory of selective elimination, and, after urging that there is probably a selection of zygotes as well as germ-cells, agrees with great courtesy, that his results are not opposed to PEARL'S, however different the racial effects of the treatment upon fowl and guinea-pigs. Although this reasoning may bring the two types of result into rational compatibility, it does not get over the fact that PEARL'S results give no evidence of germinal modification, while STOCKARD'S do. PEARL swings his discussion away from the main topic of the origin of germinal modification to the applicability of his theory, but he evidently admits that STOCKARD'S results show inheritance of defects. "STOCKARD'S F_2 and F_3 results indicate that though the untreated F_1 animals from alcoholists may appear normal, they really are somewhat defective." (PEARL 1917, p. 297.) If this difference between the fowl and guinea-pigs is admitted, no assumption of a selective elimination in the guinea-pigs, taking effect either upon the zygotes or germ-cells, can make the two experiments give supplementary results on the basic problem of inheritance. As a matter of fact, the results from the guinea-pigs do not give the slightest necessity for the assumption of any genetically selective action on the part of the alcohol. To be sure, there is nothing incompatible with this assumption, but there is little justification for making an assumption to account for the results in one generation, while another assumption is necessitated by similar results in a later generation. In the first generation the falling off in numbers may be due to the direct action of the alcohol eliminating germ-cells or zygotes; in the later generations, in the absence of alcohol, another cause must be sought for this same falling off in numbers. The sort of evidence upon which PEARL bases his theory of a selective action, namely, a falling off in numbers followed by a superiority in the survivors, is entirely lacking in the guinea-pigs, where a falling off in numbers is followed by a falling off in numbers and inferiority in quality.

In contrast to both the guinea-pigs and fowl, the rats give evidence that processes of selective elimination and germinal modification are both affecting fertility. There is a selective elimination of infertile germplasm by the suppression of whole litters through the reaction of alcohol upon the parents themselves, with the result that only the germ-plasm with the power to produce larger numbers of litters is passed on to the next generation. Beside this, alcohol has acted directly upon the germcells, causing a modification in the germinal material that results in smaller litters, and this modification appears to be inherited by later generations.

CONCLUSIONS

This paper presents evidence in support of the following conclusions:

(1) The treatment of white rats with alcohol by the inhalation method tends to reduce the size of litters. A reduction in the neighborhood of 10 percent of the respective controls appears when the daily dose was small and when it was maximum, in the litters from treated rats as well as in rats whose parents or grandparents were treated. The numbers are not large enough to make this reduction statistically significant when each generation is considered by itself, but when all the generations are taken together, the difference between the test and control averages is significant.

(2) Given daily in maximum doses alcohol reduces the number of litters produced by the treated rats. Judging by the number of litters produced by their controls, this reduction is in the neighborhood of 65 percent. The treatment of rats from treated parents made a smaller reduction (35 percent) in the number of litters than when the treatment was confined to one generation. In contrast to this, rats whose parents or grandparents alone were treated produced from a third to a half as many again as the controls.

(3) These conclusions may be interpreted as due, first, to an inherited modification that reduces the size of litters, and second, to the selective elimination of germ-plasm bearing factors detrimental to litter production through the immediate reaction of alcohol upon the treated rats. Thus alcohol appears to modify fertility in two different ways, each working independently, and, in the generations after the first, in opposite directions.

(4) In so far as these results show a reduction in the total output, they agree with those of STOCKARD on guinea-pigs and PEARL on fowl. Further than this the percent reduction in litter size in the different generations is closely alike for rats and guinea-pigs, while the results given by the numbers of litters agree with those of PEARL in showing immediate reduction in numbers, followed by a superiority in the survivors.

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THE RESULTS OF SELECTION WITHIN PURE LINES OF *PESTALOZZIA GUEPINI* DESM.[1]

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INTRODUCTION

Since JOHANNSEN'S classic experiments on beans led him to formulate his theory of the multilinear composition of species and the inefficacy of selection within any of the lines, several workers have studied the effect of selection within pure lines of various other organisms. No apology need be offered, however, for this extension of the work to one of the asexually reproducing Fungi Imperfecti, for the peculiarities of the material make possible a quite different experimental attack. Inasmuch as the literature of the selection problem has recently been thoroughly reviewed by JENNINGS (1916, 1920) and others, only those papers will be considered here which have special bearing on the present investigation.

One of the most noteworthy of such contributions is that of JENNINGS (1908) on Paramecium. In a carefully planned and executed series of experiments in which numerous selections were made, JENNINGS was unable to detect any effect of selection. This was among the first of the considerable series of investigations which appeared to bear out the JOHANNSEN theory.

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[1] Papers from the Department of Botany of the UNIVERSITY OF MICHIGAN, No. 186. A portion of this investigation was carried out at Kisaran, Asahan, Sumatra; the remainder in the Botanical Laboratory of the UNIVERSITY OF MICHIGAN.

The work of EWING (1914 a, 1914 b, 1916) is of particular interest because of the large number of generations during which he practiced selection. He studied an aphid, *Aphis avenae*, and found that selection, even for eighty-seven generations, was totally without effect.

The work of HANEL (1908) has received much attention and adverse comment. HANEL concluded that selection was without effect on Hydra, but it is doubtful whether the experimental results justified the conclusion. More recently the status of HANEL'S work has been discussed and the experiments have been repeated by LASHLEY (1915, 1916). The latter worker took account of the sources of error in HANEL'S experiments and carried out so excellent an investigation as to leave no doubt that selection within the genus Hydra is ineffective.

A number of other investigators have secured results which substantiate the work of JOHANNSEN. Some of them have made careful investigations which are of considerable value. In other cases the results of loosely planned and brief experiments have been recorded. For one to believe, because selection for one generation within a line has had no effect, that selection is therefore in all cases ineffective, requires a very sanguine temperament. There was ground for the protests of PEARSON (1910) and HARRIS (1911) that the pure-line theory had been too readily accepted before adequate evidence had been presented. However, all in all, a considerable body of data supporting JOHANNSEN has been secured, some of it exceedingly hard to refute. Until recently the results of all investigations seemed only to add more evidence in support of the theory.

Within the last few years, however, more and more conflicting evidence has been amassed, partly through critical analysis of the older data, and partly by new experimentation. JOHANNSEN'S experiments have been criticized because they were not carried on for a sufficiently large number of generations. By statistical analysis PEARSON (1910) found that there was some evidence that JOHANNSEN'S results really showed inheritance of somatic variations within pure lines. ROOT (1918) has discounted EWING'S results because the organism concerned is one which in nature is highly stable, its lack of variability affording at the start little ground for hope that new strains could be produced.

Several objections have been offered to the conclusions of JENNINGS from his work on Paramecium. One is that he studied size characters which are highly variable under different environmental conditions. These characters also vary with different stages of maturity of the individuals and it is admitted by JENNINGS himself that one cannot be certain that only mature specimens are being measured.

Turning now from reinterpretation of old work to the newer experimental data, we find that several studies have been interpreted as showing positive selection effects. Thus STOCKING (1915) found that it was possible to produce distinct groups by selection within certain lines of abnormal Paramecia. MIDDLETON (1915) was able to produce strains of Stylonychia, distinct in regard to rate of fission, and this almost at will. JENNINGS (1916), with much greater difficulty, detected small changes in Difflugia, which he attributed to selection. ROOT (1918) and HEGNER (1919), working with Centropyxis and Arcella, respectively, secured data in conformity with those of JENNINGS with Difflugia.

To this later work, however, certain objections have been made. STOCKING'S results were obtained with abnormal forms and it seems to have been generally concluded that normal variations might not necessarily behave in the same way. MAST (1917) secured two groups distinct for rate of fission in *Didynium nasutum*, without selection, presumably by a mutation in one group. This has some bearing on MIDDLETON'S results, and if it be argued that very frequent mutations would be needed to account for the rapid changes produced by MIDDLETON'S selections, it may be pointed out that great instability must obtain in an organism to allow of such changes even by selection.

MORGAN (1916) suggests that the selection results with Difflugia may be due to random distribution of discrete particles of nuclear material (chromidia) to the pairs of offspring. If such were the case, and if the bearers of particular hereditary characters were contained in the chromidia, one would not expect the effects of selection to be indefinitely cumulative. Moreover, the variations would necessarily be of a discontinuous type, and perhaps remotely comparable to non-disjunctional mutations or somatic mutations. The same suggestion would also apply to the results of selection in Centropyxis and Arcella. In all three cases it is likely that the variations dealt with are not of the usual continuous type.

LASHLEY (1915, 1916) has produced evidence that PEARSON'S criticism of JOHANNSEN'S work is not well founded, and that the parent-offspring correlation is not reliable as a criterion in selection work.

It must be obvious that the question as to whether variations occurring within a pure line are purely somatic and non-heritable or not, is one that is far from a definite answer. Further investigations on forms suitable for the study of the problem, wherever they may occur, are greatly needed.

Thus far Protozoa have been used in most of the critical and extensive investigations, and these organisms are not only subject to great environmental effects, but give rise to only two offspring at a time,—a serious disqualification for the most accurate results. JOHANNSEN's original material, the garden bean, was ideal from the standpoint of number of offspring, but, reproducing sexually, it offered a possibility of Mendelian segregation obscuring the true fluctuation which it was desired to study. The Fungi Imperfecti seemed to afford an ideal material, combining the advantages of numerous progeny with uniparental, non-sexual reproduction.

REASON FOR THE CHOICE OF PESTALOZZIA

Pestalozzia Guepini Desm., one of the Fungi Imperfecti, was chosen by the writer as a species unusually well suited for use in the study of the selection problem. The species is polymorphic, containing a number of different strains, so that it is not unlikely that new strains are being formed at the present time. It is very easily grown in culture in the laboratory and does not require tedious, special methods. Sporulation occurs readily in culture, and development from the spore to the fruiting stage requires only a few days, so that generations can be grown in rapid succession. Spores are produced in enormous numbers so that more than enough are always available for observation. They possess two characters that are capable of accurate measurement, and a change in color at maturity enables one to tell with certainty which spores are mature and which immature. Size characters may therefore be studied without fear that forms apparently small are only immature. In addition to these advantages the spores are produced, as in all Fungi Imperfecti, entirely asexually, so that there is no possibility that the results are influenced by sexual phenomena.

Pestalozzia Guepini Desm. is widely distributed in the eastern tropics, where it causes the gray-blight disease of tea, and leaf-spot diseases of cocoanut, betel nut palm, African oil palm, Para rubber, and doubtless many other wild and cultivated plants of those regions. The species contains a number of forms which differ more or less markedly from one another in morphological characters. Some of them are likewise supposed to be restricted to particular hosts. Strains occurring in regions far removed from areas known to contain *Pestalozzia Guepini*, and on hosts not previously reported for this fungus, have been described as new species. This is only natural, for the species was originally not closely defined. The new species however, are even less perfectly described than *P. Guepini*, so that one gains nothing by using their names. For the purposes of this study the concept of *P. Guepini* employed is that given in a preliminary study of the strains of the species by LARUE and BARTLETT (1922).

In this investigation the authors found that from thirty-five isolations of Pestalozzia from the Para rubber tree, the cocoanut palm, the African oil palm, the betel palm, and the tea plant, at least fourteen strains, distinct in regard to morphological characters, could be distinguished. More minute morphological studies would in all probability have resulted in the division of some of these strains into smaller distinct groups. Cross inoculations might possibly have revealed variations in regard to hosts infected, and physiological studies would almost certainly have shown that some isolations differ physiologically from others in the same morphological group. The large number of forms found within the comparatively limited territory on the East Coast of Sumatra, from which the cultures were secured suggests that an equally large number of strains might be found in other regions, many of them doubtless distinguishable from those already studied. The formation of at least one new strain by mutation during the period covered by the present investigation of the effect of selection leads to the belief that other strains are now being formed in nature and that *P. Guepini* is a species in what may be called a plastic condition. In this respect it is comparable to the organisms studied by other recent workers who have sought to find a clue to the process by which evolution proceeds.

Only the spores of the fungus were studied minutely by LARUE and BARTLETT (1922) in the investigation of variability in *P. Guepini* mentioned above. The vegetative characters and growth habits also show considerable variation but these are much more limited in range and more difficult to measure than the quantitative characters of the spores, and therefore were not utilized. For selection studies also, the spore characters were chosen as most suitable though other characters might have given equally satisfactory results had they been more easily measured.

The spindle-shaped spores of *Pestalozzia Guepini* are composed of five cells of which the three central ones are smoky or black at maturity, while the other two are hyaline. The distal hyaline conical cell normally bears at its tip three slender, unjointed, hyaline appendages which diverge so that their tips are widely separated. Spores are occasionally found which contain three or four cells, and others which bear two appendages or even only one, but these are apparently aberrant forms. Thus far no strain has been found which is constant for any of these peculiarities, and in the cases where the aberrant spores have been tested, they have produced normal progeny.

The total range of mean length of spores for all the strains of *P. Guepini* isolated by LaRue and Bartlett (1922) was from 19.9μ to 28.3μ. Figure 1 shows the polygons of variation in spore length for a few representative strains. Mean appendage lengths of the strains ranged from 10.9μ to 30.0μ. The polygons for variation in appendage length are shown for six representative isolations in figure 2. More complete data, which cannot be reproduced here, are given in the paper already cited. In view of the large number of strains which exist within the species, this fungus is a very favorable organism for use in making an attempt to develop still other strains by the selection of variant spores.

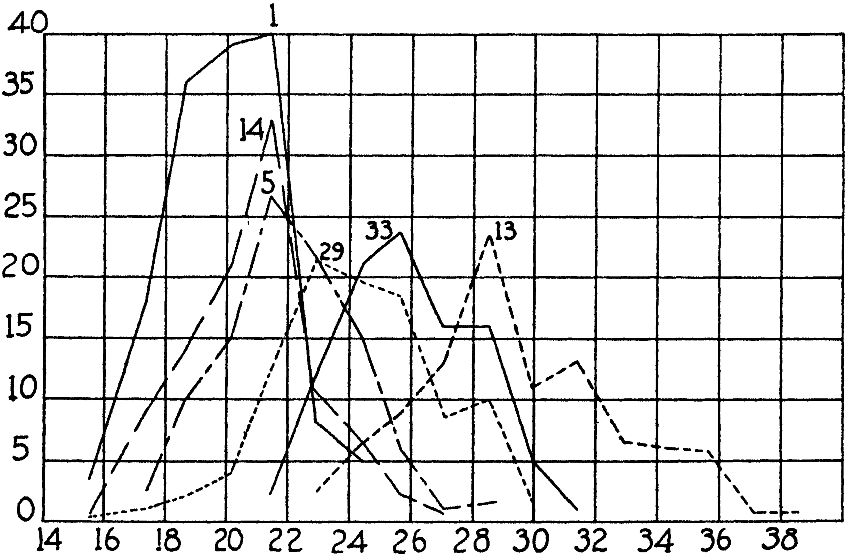

Figure 1.—Polygons of variation in spore length for six strains of *Pestalozzia Guepini*. The ordinates are percentages; the abscissae, lengths in μ.

It is essential that any organism which is to be employed in the study of the selection problem be easy to cultivate and in this respect *P. Guepini* is admirable. It will grow readily and rapidly on almost any common nutrient agar and can also be grown on sterilized leaves, twigs and fruits of its common hosts. In Sumatra usually only about four days time was needed to develop the fungus from spore to the fruiting condition. In Michigan a slightly longer time was needed to secure spores even when the fungus was kept at a temperature approximately the same as that of Sumatra. Strong light appears to inhibit the growth of the young mycelium, but exposure to light after the mycelium is about three days old seems to hasten the production of spores.

When sporulation begins the spores are developed very rapidly and in enormous numbers. This is a characteristic exceedingly valuable in biometrical studies, since the variates are produced under as nearly identical conditions as may be found anywhere. By measuring a sufficient number of such spores one can get the whole range of variability induced by the reaction of a given set of environmental conditions with the hereditary characters of the organism. In an organism which produces offspring slowly a number of fluctuations in environment must necessarily take place before a number of individuals sufficient to give a reliable mean is produced. This complicates the situation greatly and the means of two groups of organisms so produced are less readily comparable than those developed in an organism such as Pestalozzia.

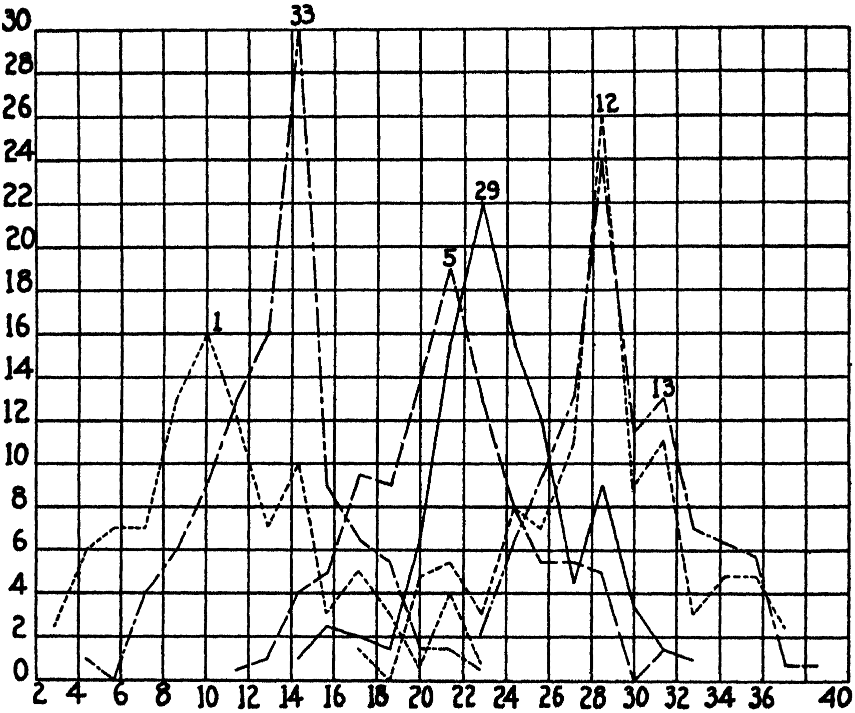

FIGURE 2.—Polygons of variation in length of spore appendages for six strains of *Pestalozzia Guepini*. The ordinates are percentages; the abscissae, lengths of appendages in μ.

Mention has already been made of the dark color of the three central cells of mature spores of *P. Guepini*. This color is a valuable index of the age of the spores because it does not fully develop until the spores have reached their full size and the appendages have been fully formed. One can always recognize mature spores at a glance and is thus able to reject all immature stages of development. This completely obviates one great difficulty usually found in the study of size characters, that of determining which individuals are really small, which only immature. This difficulty seems insurmountable in such forms as Paramecium, but in Pestalozzia, as in Difflugia, the nature of the organism allows size characters to be observed without fear of complications from this source.

A further word of explanation may be desirable with regard to the assumed absence of sexual reproduction in Pestalozzia. As has been stated, it belongs to the group of Fungi Imperfecti. Many of the species in this miscellaneous assemblage have been found to have other stages in their life-history which have enabled them to be classified under other groups. With few exceptions these other stages of Fungi Imperfecti have shown them to be degenerate members of the Ascomycetes. Many Ascomycetes have both the ascus stage, which follows a sexual fusion, and an asexual, conidial stage. It is commonly assumed that the conidial forms, which make up the Fungi Imperfecti, either have, or have had at some time, a stage with a higher spore form. Whether Pestalozzia now has an unknown ascus stage is not so important, as is the probability that if it had a sexual stage at all, it would be indicated by the appearance of a readily recognizable ascus stage. No such form has ever been observed in the hundreds of cultures grown in this series of experiments and it is reasonably certain that it never appeared. From the data gained from the study of analogous forms we can safely assume, (1) that the conidia of Pestalozzia are entirely asexual, (2) that if a sexual stage appears it will result in the production of an ascus form and therefore will be easily detected, and (3) that we have no need to fear that obscure nuclear recombinations are complicating the results secured.

The unique combination of characters possessed by Pestalozzia and discussed above renders the organism an unusually suitable one for use in study of the results of selection within vegetative lines. Because the fungus was so well adapted for this use and because no similar material had ever been used for this purpose the author was led to undertake the investigation here described. Presented in review, the characters which make Pestalozzia specially desirable for such use are: (1) The presence of numerous distinct strains within the species; (2) the ease with which it is grown in culture; (3) the rapidity with which consecutive generations may be produced; (4) the availability of at least two easily measurable independent characters; (5) the rapidity with which spores are produced and the enormous numbers of spores produced, which enable one to secure significant statistical constants for each generation; (6) the dark coloration of the three central cells of the spore which appears only at maturity, and serves as a criterion for the elimination of mere growth stages; (7) the total absence of any sexual form of reproduction.

EXPERIMENTS

Methods and conditions

Since the aim of the investigation was to study variations of genetical significance, every reasonable attempt was made either to remove variations in environment or to provide controls for them. All the cultures used were grown on agar in tubes, and under ordinary laboratory conditions. Aside from the fact that it would be extremely inconvenient, if not impossible, to control a selection experiment in which the organism was grown as a parasite on its usual host, such a procedure would doubtless offer more variations as to food supply, etc., than would growth on culture media. Wollenweber (1914) has shown that organisms which are facultative parasites show as normal development in culture as on their usual hosts.

That fungi, especially Saprolegnias, may be exceedingly sensitive to variation in food supply has been shown by Kauffman (1908) and Pieters (1915). However it is not believed that Pestalozzia is so exquisitely sensitive to such variations as are some other fungi. In any event the variations thus introduced into this work are doubtless much smaller than those which of necessity apply to any other organism which has been used in the study of selection up to the present time.

The nutrient solution was all made up before the experiment was begun. Tender twig tips and young leaves of *Hevea brasiliensis* were boiled in water and a quantity of brown sugar made from the sugar palm (*Arenga saccharifera*) was added. The decoction was strained, concentrated by boiling, and after thorough mixing to secure uniformity, was poured into flasks which were plugged with cotton stoppers and sterilized.

The single lot of medium thus prepared was stored and used throughout the experiments until the writer's return to the United States. (A supply of the medium was brought to America but was lost in transit, so that it was necessary to supply a substitute, as will be noted later.) For growing the cultures nutrient agar was made up according to the following formula:

Hevea decoction,	250 cc
Water,	3750 cc
Agar-agar,	120 gm

The medium was very thoroughly mixed before being put into tubes, and the same amount was placed in each tube. In making cultures care was taken that all the tubes of a given generation were poured from agar of the same lot.

The cultures were not kept in a constant-temperature chamber but the range of variation in temperature and humidity is not great in the east coast region of Sumatra. For the greater part of the year the daily change in temperature is from about 23° C to about 32° C. The total range of fluctuation for one five-month period of the selection experiment was from 19.4° to 33.9° C. During the same period the moisture content of the air varied from 52 percent to 100 percent with a usual daily range of approximately from 60 percent to 100 percent. In general the climate of Asahan varies so little from day to day, or from month to month, that it would be hard to find any place more uniform. This uniformity allows cultures to be carried on indefinitely without danger of extremes of heat or cold such as have ended various experiments of other investigators.

The selection experiments were made according to two plans. In the major part of the work the line of descent was determined wholly by divergence in progeny means without regard to the visible characters of the spores chosen as generation parents. In other words, the direction of selection in the chief experiments was guided by the *performance* of particular spores, rather than by their *appearance*, but the method was checked by parallel experiments in which the usual basis of selection, namely, selection of visibly divergent individual spores, was employed.

The characters concerned were length of spore and length of spore appendage. That these characters are heritable to a very considerable degree is shown by the fact that strains, distinct for either spore length or for length of spore appendages, have been isolated, and that these strains remain distinct from generation to generation in spite of fluctuations in environmental conditions. The heritable differences between the different strains are very considerable as may be seen from figures 1 and 2, and these differences make them especially promising for use in a study of the results of selection.

The number of cells in the spore and the number of appendages per spore might appear to be characters suited for study but this was soon found not to be the case. The number of cells in the spore shows little variation; the smallest number found in observing hundreds of thousands of spores was three, and the largest number seen was nine. In two cases where spores having one more than the normal number of cells were selected it was found that the spores produced by the mycelia from these spores were entirely normal in regard to cell number.

The range of variation in number of spore appendages is even less than that in cells. The greatest number recorded from all observations made was four and the smallest number, one. Three different spores were isolated which bore only one appendage each. However, when these spores germinated and the mycelia from them produced spores, the latter were found to have the normal three appendages. Another spore bearing two appendages was isolated with the same result, and still another bearing four appendages gave progeny which showed no sign of this abnormality. Apparently variation in cell number and spore appendage number are not greatly different in behavior from sundry other abnormalities which appear from time to time in Pestalozzia, none of which appears to be hereditary.

Selection of spores according to their progeny

The method of procedure in selecting spores according to their progeny was as follows: A single spore culture was made from a given strain. When this culture produced mature spores agar plates were poured with various dilutions of these spores. A mass of spores was taken from the culture with a platinum wire and mixed with about 10 cc of sterile water in a sterile tube. From this water suspension of spores two loops were put into a tube of melted agar and thoroughly mixed. Two loops of this agar were then mixed in the next tube; and again two loops were used to inoculate the third tube. The agar from each tube was then poured into a sterile petri dish, and the dish was covered with a bell jar. When the spores germinated the agar plates were examined and spores were cut out from the most suitable ones and put into separate tubes of Hevea agar. Microscopic examination was made in each case to make sure that only one spore was used in inoculating each tube. On this account it was found more convenient to use a 1-percent beef-extract agar instead of Hevea agar for pouring the plates. It was not always possible throughout the experiments to regulate the number of spores in the poured plates. The third dilution plate was the one from which the spores were usually taken, but whenever this contained too few spores to make a full set of cultures for a given generation, the second dilution plate was used instead.

All the tubes contained agar made in one lot, tubed and sterilized at the same time, and stored under the same conditions. The transfers of the twenty single spores were made one immediately after the other, so that the time of transfer of each was so nearly the same as to admit of no reasonable assumption that this variation could be of effect. The twenty tubes were put into one rack so that all might be subject to the same general environmental conditions.

When the mycelia had all grown and the spores were mature, 100 spores of each culture were measured and the mean spore length, or mean appendage length, as the case might be, was computed. By trial it was found that 100 measurements were sufficient to secure a smooth distribution curve and a mean with a satisfactorily small probable error, so with some exceptions, where a few more spores were taken, one hundred from each culture were measured. Typical distributions are shown in table 1.

TABLE 1

Typical frequency distribution of measurements of spores and spore appendages from different cultures of Pestalozzia Guepini. Measurements in μ.

SPORE LENGTHS				SPORE APPENDAGE LENGTHS			
Length	Experiment 1 Plus group Generation 3 Culture 3	Experiment 1 Plus group Generation 3 Culture 8	Experiment 1 Minus group Generation 4 Culture 3	Length	Experiment 1 Minus group Generation 6 Culture 1	Experiment 2 Plus group Generation 1 Culture 1	Experiment 2 Plus group Gen. 1 Cul. 15
19.8	1	2	3	10 8	2		
20.7	1	2	3	12 6	2		2
21 6	4	5	8	14 4	3	3	1
22.5	8	9	11	16 2	8	5	7
23.4	11	17	15	18 0	15	11	12
24.3	12	19	17	19 8	17	12	15
25.2	19	14	15	21.6	16	15	18
26 1	14	12	12	23 4	13	17	14
27.0	8	9	6	25 2	10	14	10
27 9	8	4	3	27.0	7	14	8
28.8	8	4	2	28 8	5	5	6
29.7	2	1	2	30.6	2	3	3
30.6	2	2	2	32 4		1	3
31.5	1		1	34 2			1
Means	25.54±.15	24.79±.15	24 53±.16		21.22±.21	22.81±.27	22.42 ±.31
Standard deviations	2.18	2.18	2 38		4.61	3.96	4.57

After the twenty cultures were measured the one with the greatest mean spore length was chosen as the starting point for plus selection. Similarly the culture with the smallest mean spore length was taken as the beginning of the minus selection. Another culture, which had a mean spore length as nearly midway between the other two as could be found, was chosen as the origin of an intermediate line to be carried on from generation to generation without selection.

From each of the chosen cultures plates were now poured. When the spores had germinated, ten single spores were cut out from the plates made from the plus-selection culture and the same number from the minus-selection culture. From the intermediate culture only one single-spore culture was made. See figure 3.

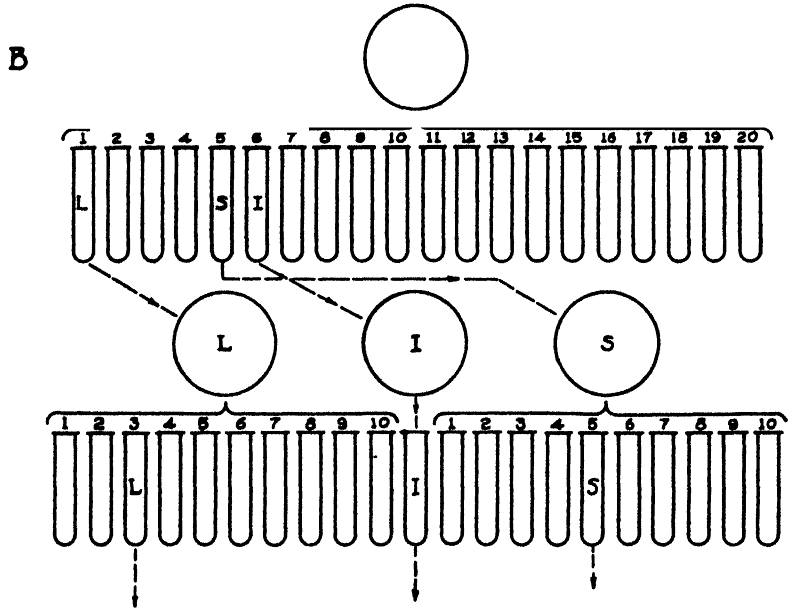

FIGURE 3.—Plan of method of selection used in experiments 1 and 2. A represents the parent culture; B, the agar plate poured with a dilution of spores from A; C, the daughter cultures grown from spores from B, of which L has the highest mean value for the selected character and is chosen as parent of a plus-selected line; S has the lowest mean value for the selected character and is selected as parent of a minus-selected line; and I, with a mean as nearly intermediate between those of L and S as possible, is used as parent of an unselected intermediate line; D, the plates poured from cultures L, S, and I; and E, the daughter cultures grown from spores isolated from the plates in D. L and S are again selected as parents of the plus and minus lines, respectively, and I is carried on without selection.

All the plates from the three different cultures were poured on the same day, and from agar of the same lot. All the spore transfers were made on the same day and all were kept in the same rack under the same environmental conditions. Whatever the influences of environment were they would act on all the cultures in the same way, and such variations as might occur between cultures would therefore be ascribed to heredity.

Some variation in time of sporulation was always found but no definite correlation was noted between the characters studied and the relative rapidity of development. It was found that the difference in size between the spores first developed in a culture and those developed several days later was not significant when compared with the differences which were usually found between different cultures.

TABLE 2

Comparison of spore appendage lengths of early and late spores from the same culture. (Strain 29, experiment 2, generation 17, plus-selected group, culture 3.) Lengths in μ.

LENGTHS	EARLY SPORES	LATE SPORES
9.0	1	2
10.8	1	9
12.6	4	13
14.4	16	14
16.2	16	18
18.0	28	23
19.8	20	12
21.6	8	6
23.4	2	3
25.2	2	
27.0	2	
Total	100	100
Means	18 16±.216	18 03±.234
Standard deviations	3 15±0 15	3.36±0 16
Difference of means	0 13±0 32	

This fact is shown in table 2 where distributions of measurements of early and late spores from the same culture are given. The early spores were taken when only a few had been formed, the late spores were the last of which the time of formation was definitely known. It should be noted that all the spores measured here, as in all other parts of this investigation, were mature normal spores, being fully septate and having three dark central cells. The difference between the mean appendage length of early and late spores is $0.13 \pm 0.32\mu$. From SHEPPARD'S tables (PEARSON 1914) it may be found that the chances are fifteen to one that this difference is due to random sampling. Accordingly it may be assumed that the two sets of measurements are parts of the same frequency distribution. Thus it appears that one may safely measure either early or late spores of a culture without fear of introducing serious errors. In practice the cultures were not measured until a vast number of spores had been produced and the sample was then taken by drawing a sterile platinum wire over the fruiting surface of the culture from the bottom of the agar slant to its top. In this way a random sample was doubtless secured.

The sample was now mounted on a clean slide on which a drop of a weak solution of magenta-red in 40 percent alcohol had been placed. The spores were mixed in the solution by stirring with the platinum wire, covered, and the excess of solution removed with blotting paper after which the spores and appendages were clearly defined in the remaining solution. The appendages and the hyaline cells at either end of the spores were stained deeply by the magenta-red, so that both spore length and length of appendage could be measured accurately. The solution did not cause plasmolysis or shrinking of either the spores or the appendages.

Duplicate measurements were avoided by measuring all spores encountered in a path across the slide just below the upper edge of the cover-glass and in another such path just above the lower edge of the glass. The chances that any spore was encountered twice were thus rendered exceedingly small.

When the means of the ten cultures of the plus group and those of the minus group had been measured in the manner described above, the cultures with the longest and shortest means were selected to continue the plus and minus selections respectively.

As contrasted with the selection methods of other investigators, it will be seen that only the range of variation happening to occur in a random sample of ten variates is made available by this method. It may sometimes happen that ten variates chosen at random will all fall near the modal condition, but ten is a large enough number so that in general a random sample of this size is likely to spread over a fair proportion of the range of variation. To have made more than ten cultures in each line of descent in each generation would have required an impossible amount of labor in measuring. A larger number, as 100, for instance, would have insured the detection of a greater number of extreme variates, but would also have vastly increased the likelihood of selecting sporadic mutations rather than extreme plus and minus variates in the range of continuous variation. From one point of view the method might be looked upon as an inefficient one, in that it surely failed to take advantage of the total range of variation in each generation.

However, when it is considered that not only is the culture from which the spore came known to possess the quality for which selection is being made, but the culture derived from each spore in the line of descent is known to possess this quality before that spore is selected as a parent, it is clear that each selection is much more significant than is usual in cases where many individuals are selected in each generation. For example, in the line selected for plus spore length, each spore chosen as a parent was known to be descended from a series of ancestral spores, each of which had the potentiality for producing a longer-spored progeny than nine other spores of its own generation, chosen at random. Similar plans of selection have been used in one or two previous investigations but not extensively nor with so full a knowledge of the behavior of both the ancestors and the progeny of the parent individuals as in the present work.

So far as the writer knows no other investigator of the selection problem has been able to establish the mean of the offspring of each selected individual as was done in this experiment. Most of the organisms which have been used in selection experiments are of such a nature as to prevent the possibility of securing a sufficiently large number of offspring, all of one age and all developed under the same environmental conditions, to establish such means. Obviously to secure as many as a hundred direct offspring from one parent Difflugia, (JENNINGS uses the term parent as applying to the individual of a dividing pair which retains the old shell), one would have to wait for just that many divisions of the parent, which would demand considerable time. The progeny being formed at different times, might be subjected to different environmental influences, so that the range of variation might be greatly increased. It is therefore not possible to know, in such forms as Difflugia, what the mean of the offspring would be were they all produced under the same conditions. When too small a progeny is secured, one does not know whether these approximate the mean value for the generation or lie at one or the other extreme of the range. Pestalozzia is especially favorable in that one can attain a full knowledge of each generation, which is not the case for most forms. Though such data are obtained only by the expenditure of a vast amount of labor it is believed they are well worth while in a serious study of so complicated a problem as that with which we are here concerned.

Experiment 1. Selection for length of spores

In this experiment the character studied was the length of the spore from the tip of the proximal cell to that of the distal cell. In figure 1, frequency polygons for this character are presented for six strains, of which No. 29 was the one used in this experiment. Strain No. 29 was secured from an isolation of fungi from the wood of a sapling of the Para rubber tree, *Hevea brasiliensis*. Previous to its use in this experiment it had been observed for eight consecutive generations in pure culture on Hevea agar. The data concerning these generations are shown in table 3.

The experiment lasted from January 1919 to June 1919 and included ten selections, all of which were made exactly according to the method described above. Through some unfavorable circumstance, one of the selections for the eleventh generation failed to grow and the experiment was thus brought to an end. The entire experiment was done in Sumatra, and Hevea agar was used for growing all the cultures.

TABLE 3

Mean spore lengths of cultures of strain 29, grown prior to the initiation of experiment 1. Measurements in μ.

GENERATION	SPORE LENGTH	RANGE OF MEASUREMENTS
1	23.6	21–29
2	21.7	16–27
3	25.6	21–30
4	22.2	17–26
5	25.1	21–29
6	25.9	23–30
7	25.5	21–30
8	24.8	21–29
9	25.95	25–40
Mean	24.5	

Figure 4 shows the greatest mean spore length in each generation for the plus selections and the least mean spore length in each generation for the minus selection; the so-called intermediate line carried on without selection is shown also. The figure thus shows the range of difference between the plus and minus selections. In case an effect of selection were present, this range should become greater. In this experiment, this is obviously not the case, since the range does not become greater. It is true that it increases at different times for a number of generations, but this increase is followed by a corresponding decrease, so that in the end no permanent change is effected. The intermediate line shows continual fluctuation from generation to generation. Similar fluctuation has been found by all workers who have made careful studies of variation in pure lines of organisms. It is presumably due to environmental influences, although the writer was unable to correlate very considerable fluctuations in the spore length of Pestalozzia with any significant fluctuations in any of the environmental factors which are easily measured. The fluctuations in the plus and minus selections appear to be due, not to selection, but to such influences as cause the variations in the unselected intermediate line. In most cases the fluctuations of the plus and the minus selections in a

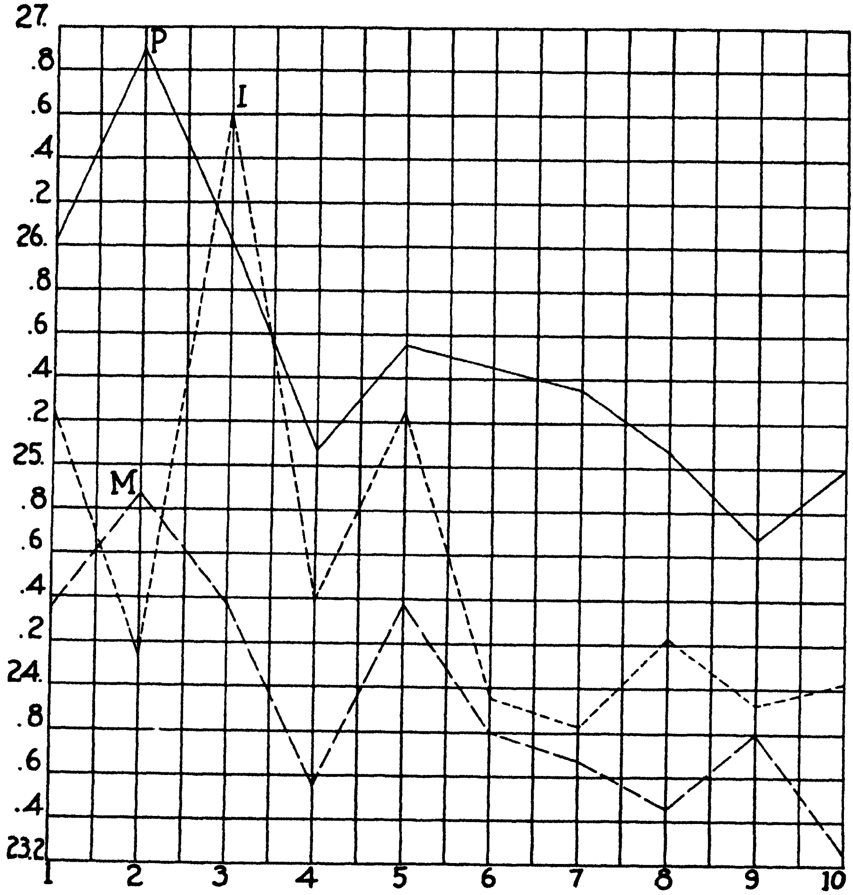

FIGURE 4.—Graph of the mean spore lengths of parent cultures of experiment 1. P represents the parents of the plus-selected line, M those of the minus-selected line, and I, those of the unselected intermediate line. The ordinates are lengths in μ; the abscissae, the generations of the experiment.

given generation parallel each other. When this is the case the deviation between the two selections is small. When the fluctuations, for any reason, do not parallel each other the deviations between the two selections may be considerably increased or considerably decreased.

From an inspection of figure 4 it is difficult to tell whether or not the parent cultures of the plus and minus strains are really different. It is absolutely essential in selection studies that all selections be significant, that is, the plus selection must be greater than the minus selection, not only apparently, but statistically. It is by no means certain that this has always been the case in previous investigations, and in many cases it is difficult if not impossible to know whether or not the differences are significant, especially where individuals are selected without knowledge of the mean of the generation.

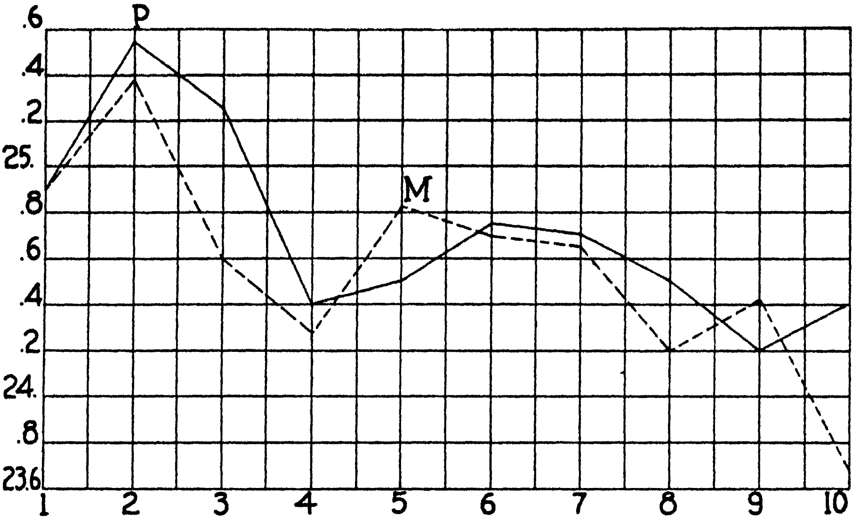

FIGURE 5.—Graph of means of spore lengths of generations in experiment 1. The plus-selected line is designated by P; the minus-selected line by M. The ordinates are lengths in μ; the abscissae, the generations of the experiment.

Pestalozzia is a very favorable organism in this respect since one can employ the mean spore length of the selected cultures and know at the same time the means of all the other cultures of that generation, as well as the mean of those means. In this experiment it was found by computing the errors of the differences between the plus and the minus selections that these differences were significant in all cases. The smallest difference found in the course of the experiment, $.900 \pm .206\mu$ in the ninth selection, is more than four times its probable error and may be considered significant.

The means of the different generations of the plus and minus lines are plotted in figure 5 and show variations comparable to those shown in figure 2. If an effect of selection had been secured the means must necessarily have shown increasing deviation one from another throughout the experiment. Such increases as they have shown have been compensated by corresponding decreases so that it becomes evident as the experiment is carried on for a considerable period that these increases and decreases of the deviation between the two selections are only the result of the chance fluctuations which take place within the two lines. It is probable that such differences would occur between any two separate lines, descended from the same parent but cultivated separately.

TABLE 4

Mean spore lengths of generations of experiment 1. Each mean was computed from the means of the cultures in its group for that generation. Each mean, except in the intermediate group is accordingly based on approximately 1000 individual measurements. Values are given in μ.

GENERATIONS	PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
1 (unselected)	24.91	24.91	24.91
2	25.54	25.38	24.12
3	25.25	24.61	26.59
4	24.41	24.28	24.38
5	24.51	24.82	25.25
6	24.75	24.70	23.94
7	24.71	24.64	23.83
8	24.50	24.21	24.23
9	24.19	24.43	23.92
10	24.41	23.67	24.03
Means of generation means based on means of all cultures in each line	24.727±0.0559	24.622±0.0407	24.552±0.126

Difference between plus-selected line and minus-selected line = 0.105 ± 0.068.
Difference between plus-selected line and intermediate line = 0.18 ± 0.104.
Difference between minus-selected line and intermediate line = 0.07 ± 0.097.

The means of each of the generations in the plus-selected line, the minus-selected line, and the unselected intermediate group, and also the mean of means of all cultures in each of these lines for the whole experiment are shown in table 4, while table 12 (in the appendix) gives the means of all cultures measured in this experiment. The differences between the lines are in themselves very small and when compared with their probable errors they are seen to be insignificant. However, had the experiment been discontinued after three selections it would have appeared that a small, but possibly significant, result had been produced by the selections. The difference between the two lines at that time was 0.267μ, which is probably more than three times its probable error which has not been computed. It is at any rate, more than three times the error of the difference between the plus- and minus-selected lines for the whole experiment.

When selection is continued for a longer time the apparent effect of selection is soon lost and the minus-selected line at times produces longer spores than the plus-selected one. The intermediate form, which is entirely unselected, shows greater fluctuations than either of the other groups, and its mean for the whole experiment is less than that of the minus-selected line. If we assume that the upward and downward swings, so prominent in this experiment, are merely chance fluctuations due to environmental influences we can understand why the intermediate line shows more fluctuation than the others, since it is represented by only one culture in each generation and its generation means are computed from only 100 measurements instead of 1000 as in the case of the other two lines.

Since the two selected lines cannot be shown to differ from one another, or from the unselected line, we can only conclude that selection has been of no avail in producing lines of *Pestalozzia Guepini* distinct for length of spore.

Experiment 2. Selection for length of spore appendages

In this experiment the same strain was used as in experiment 1, namely, No. 29. Table 5 presents the means and ranges of appendage length for the eight generations during which the fungus had been studied prior to the initiation of this experiment, and a frequency polygon of this strain is shown in figure 2.

The plan of the experiment was that explained earlier in this paper and used in experiment 1, and was rigidly followed throughout the whole series of selections which were continued for more than a year. All of the cultures were grown on Hevea agar, made according to the formula already given, from the original lot of Hevea decoction, and used with all the precautions previously mentioned. This work, like that of experiment 1, was all done in Sumatra within a few hundred yards of the place where line No. 29 was originally found.

In this experiment twenty-five selections were made for length of spore appendages, and twenty-five successive generations were grown of an unselected intermediate line also. The appendage lengths of all the parent spores are shown in figure 6. The extreme cultures of the plus and minus lines parallel each other in a remarkable manner but the differences between them are not great. In one case (generation 11) the two lines are so nearly coincident that parent cultures certainly different for appendage length, could not be secured from the plus and minus lines. The range of variation in appendage lengths did not consistently increase from generation to generation as they should if selection were effective but underwent successive increases and decreases as the two lines drew nearer together or deviated more widely. The differences between the plus-selected parent and the minus-selected parent vary from generation to generation, but are large enough to be of significance except in the one case mentioned.

Figure 7 shows the means of the generations for the whole experiment and from this figure it is at once apparent that the two lines of descent did not become more widely divergent as the number of selections increased. Instead the same upward and downward swings appear as were seen in experiment 1, but in this experiment the two lines parallel each other in these fluctuations in a surprising way.

TABLE 5

Mean lengths of spore appendages of cultures of strain 29, grown prior to beginning experiment 2. Measurements in μ.

GENERATIONS	LENGTHS OF APPENDAGES	RANGE OF MEASUREMENTS
1	24.5	20–30
2	21.7	10–27
3	23.1	16–30
4	26.7	20–36
5	25.1	20–39
6	22.4	14–39
7	24.0	16–33
8	24.1	19–31
9	21.8	16–40
Mean	23.71	

The means of the generations of each line and the mean of means of each line for the whole experiment are presented in table 6, while table 13 (in the appendix) gives the data for all the cultures grown during the course of the experiment. After examining figure 7 one is not surprised to find that the differences between the three different experimental lines are of no significance. In one case the difference is less than its probable error. Each case where the plus line has grown longer and the minus line shorter has

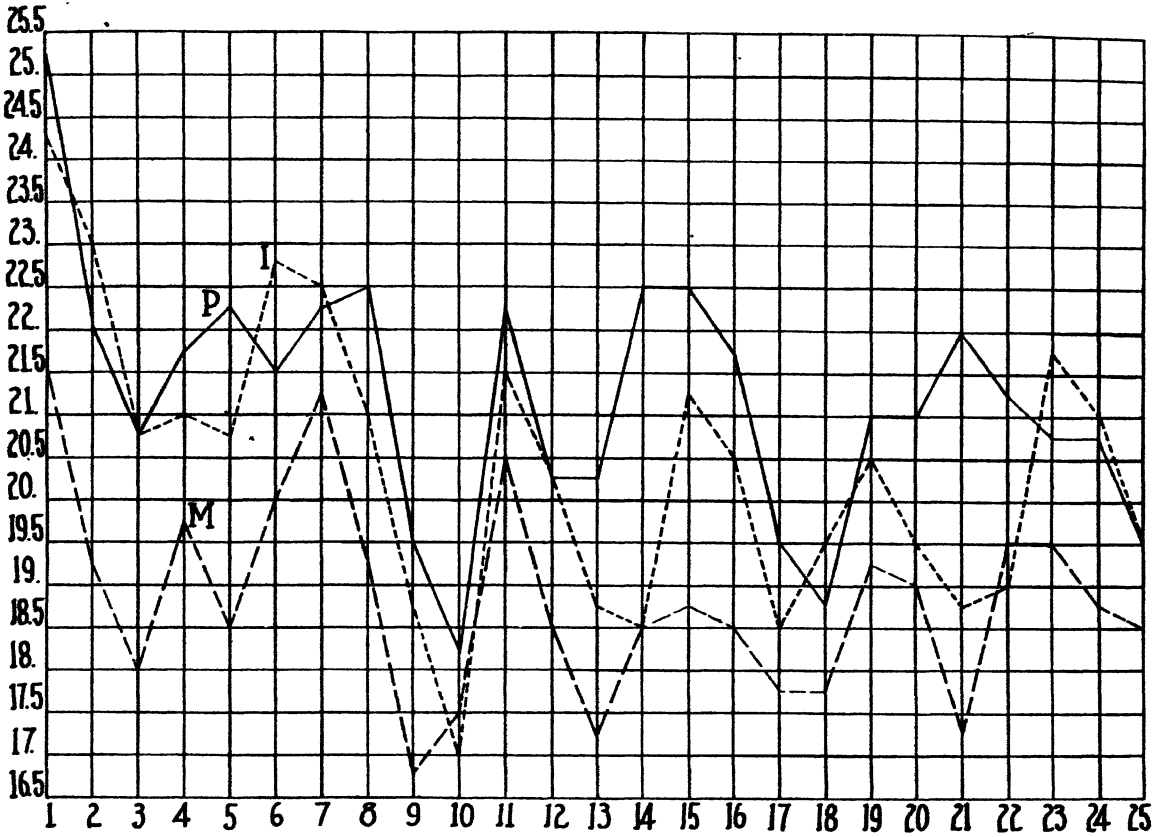

FIGURE 6.—Graph showing the mean lengths of spore appendages of parent cultures of experiment 2. P shows the parents of the plus-selected line; M, those of the minus-selected line; and I, those of the unselected line. The ordinates are lengths in μ; the abscissae, the successive generations of the experiment.

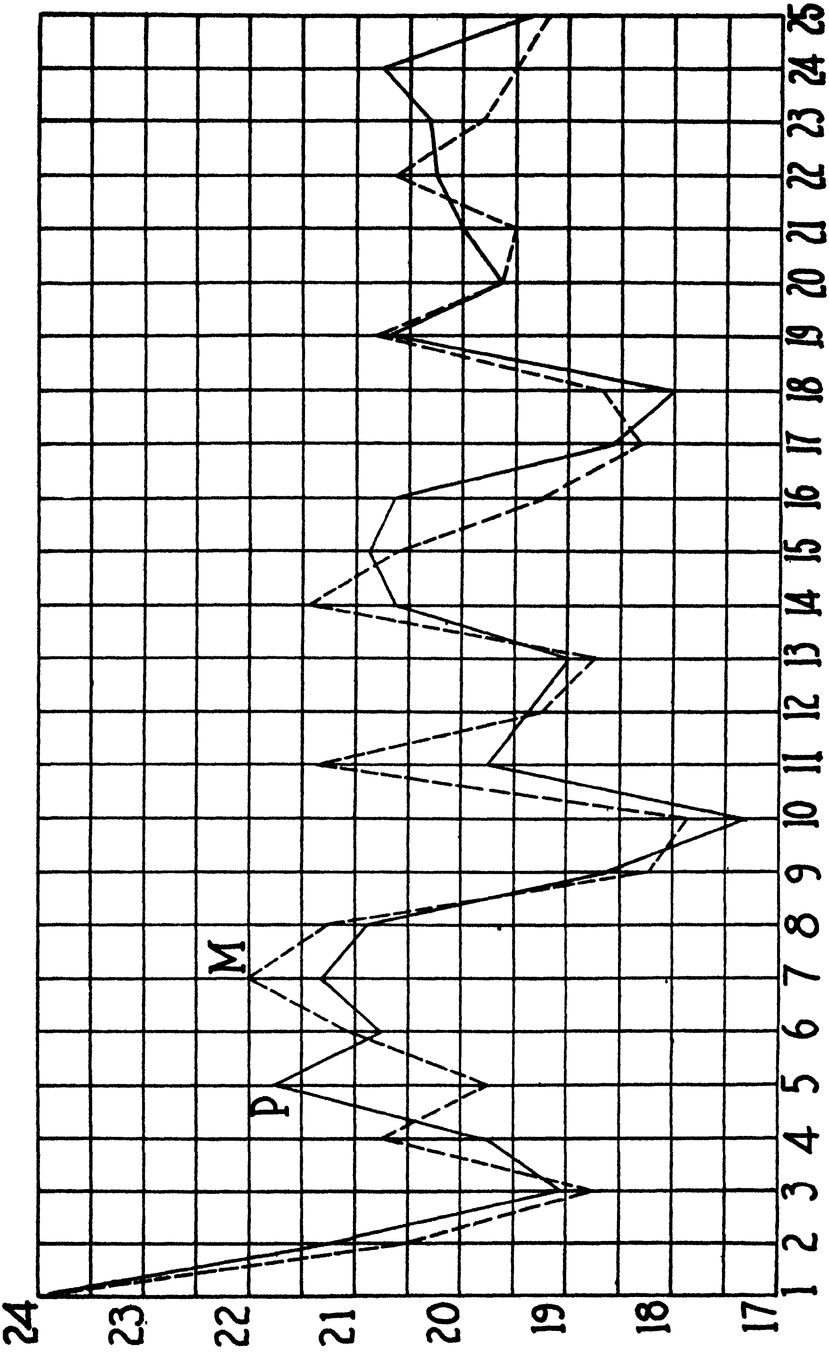

FIGURE 7.—Graph showing the means of spore-appendage lengths of the generations in experiment 2. P represents the plus-selected line; M the group of minus selections. The ordinates are lengths in μ; the abscissae, the generations.

apparently been so nicely balanced by one where the minus line has grown longer and the plus line shorter, that the result has been an almost absolute identity of the two lines as regards length of spore appendages.

TABLE 6

Mean appendage lengths of generations of experiment 2. Each mean was computed from the means of the cultures in its group for that generation. Each mean, except in the intermediate group, is based on approximately 1000 measurements. Measurements are given in μ.

GENERATIONS	PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
1	23.94	23.94	23.94
2	21 22	20.54	23.04
3	19.06	18.74	20.78
4	19.66	20.74	20.93
5	21 78	19.73	20.78
6	20.77	21.08	22.63
7	21.32	21.99	22 41
8	20.88	21.22	20.98
9	18 61	18.23	18.83
10	17.33	17.87	17.05
11	19.71	21.37	21.39
12	19.42	19.28	20.14
13	18.99	18.74	18.67
14	20.66	21.42	18.59
15	20.90	20.63	21.24
16	20 61	19.26	20.47
17	18.56	18.34	18.62
18	17.98	18.70	19.44
19	20 68	20.83	20.47
20	19.62	19.62	19.42
21	20 03	19.53	18.65
22	20.20	20.65	19.04
23	20.34	20.29	21.83
24	20.74	20.02	21.06
25	19.31	19.22	19.62
Means of generation means based on means of all cultures in each line	20.394±0.0844	20.223±0.0774	20 502±0.227
Standard deviations	1.908±0.0598	1.746±0.050	1 681±0 160

Difference between plus-selected line and minus-selected line=0.171±0.115.
Difference between plus-selected line and unselected intermediate line=0.108±0.242.
Difference between minus-selected line and unselected intermediate line=0.279±0.239.

The minute scrutiny of each generation in the course of this experiment and of experiment 1 made it possible to detect at once the appearance of any aberrant form by mutation. It is desirable that such control be kept in all selection studies since it is not improbable that in some cases small mutations have arisen in the course of selection experiments, which have not been detected and have produced effects attributed to selection. In this experiment in the minus line of generation 14, culture 5, a distinct mutation appeared which had a greater length of spore appendages than any other culture encountered in the whole experiment. The spores themselves were also longer, much more slender, and lighter in color than those of any other culture seen during the whole range of my experience with Pestalozzia. This form has since remained distinct through several generations and will be studied further at a later date. It is mentioned here merely to show the possibility that such mutations may account for some of the results which are apparently due to selection. In the present instance the mutation occurred in a plus direction but in the minus line and so could hardly have escaped attention, but had it occurred in the plus line, had it been somewhat less striking, and had it occurred in a less carefully controlled experiment it might have escaped detection, and have greatly confused the results of that experiment.

That the new form was not a contamination from outside instead of a mutation, was made practically certain because no such form had ever been isolated from the surrounding country; because all culture work was carefully done and the usual contaminating organisms were rarely found in the cultures; because Pestalozzia is not a fungus which is likely to be found as an air-borne contamination in cultures; and finally, because blank plates were poured in many generations without ever securing any growth of any organism.

Experiment 2, carried on for more than one year, through twenty-five generations, involving nearly 500 different cultures, and 50,000 spore measurements, should have been a sufficiently thorough and long-continued study to demonstrate any effect of selection which might have appeared. Since no such effect has been demonstrated we can only conclude that the result of this experiment agrees entirely with that of experiment 1 and that it is not possible to produce lines distinct for lengths of spore appendages by even long-continued selection.

Selection of spores according to visible characters

The selection of spores according to their appearance was now undertaken as a check on the results of the previous selections. On account of the small size of the spores of Pestalozzia considerable difficulty was experienced in finding a method by which the measured spores could be isolated. Finally a special method was devised which has been described in the Botanical Gazette (LARUE 1920).

In this method a selecting device was used which consisted of a brass cone threaded at one end, and turned into a small tube at the other, the end of the wall of the tube being made thin so as to form a sharp cutting edge. The device was screwed into the nosepiece of a microscope in place of one of the objectives. The spores to be examined were mixed in a sufficient dilution in a tube of 1-percent LIEBIG's beef-extract agar and sterile slides were spread with a thin layer of this agar. When the agar cooled the spores were firmly fixed in a thin transparent agar matrix and could easily be located and measured under the microscope. When a spore of the desired size was located it was carefully centered in the field of vision and the nose-piece of the microscope was turned so as to place the selecting device, of which the tip had just been sterilized by flaming with a gas or alcohol flame, immediately above the spore. In a good microscope, made so that the objectives center properly, this is an entirely mechanical process requiring no skill on the part of the operator. The microscope tube was now lowered until the sharp tube entered the agar and cut a ring completely around the spore. The agar disk containing the spore was now examined to see that only the chosen spore was contained in it, and then lifted with a flattened platinum wire and transferred to a tube of nutrient agar.

The agar used for imbedding the spores on the slides was filtered very carefully to render it as transparent as possible so that it might not interfere with a clear view of the spores. After selection the spores were grown in Hevea agar of the same composition as that used in the former experiments. However, after five selections had been made the cultures were transferred from Sumatra to Michigan, and the supply of Hevea decoction taken with them having been lost in transit, prune-juice agar was used for the remaining cultures of the experiment. The prune agar, which gave satisfactory results, was made by adding 2 percent of agar-agar to a decoction of prunes. Since only one lot was made, and used for all the cultures the exact proportions are of no special significance in the experiment, and are therefore omitted. Care was taken, however, to see that the medium was as uniform as possible for all the cultures.

Experiment 3

The line used for this experiment was No. 17, an isolation made from a leaf-spot of a seedling of *Hevea brasiliensis*, which had been grown in culture and measured for nine generations prior to the initiation of the experiment. The variation of the strain during these generations may be seen in table 7.

The character selected in this study was length of spore. Spores of the last culture shown in table 7 were mounted in agar on slides and measured. The longest spore found was selected as the parent of a plus-selected line, and the shortest spore encountered was chosen as the parent of a minus-selected line. When sporulation took place in the resultant cultures the spores were measured and the mean spore lengths of the cultures determined precisely as in experiments 1 and 2. Spores from each culture were now mounted and measured, the longest found in the plus culture being selected to continue the plus line, and the shortest found in the minus culture being taken as parent of the next generation of the minus line.

TABLE 7

Mean spore lengths of cultures of strain 17, grown prior to the selections in experiment 3. Measurements in μ.

GENERATIONS	SPORE LENGTHS	RANGE OF MEASUREMENTS
1	22.0	17–24
2	21.1	17–23
3	21.2	19–24
4	23.4	20–29
5	22.3	19–29
6	22.5	17–26
7	23.3	19–27
8	23.5	20–28
9	23.9	20–28
Means	22.58	

The selections thus begun were continued until ten had been made when the experiment was discontinued. No attempt was made to make the selections in the two lines at the same time. Thus the cultures in the two groups usually developed under somewhat variable environmental conditions so that a given selection is not precisely comparable with the selection of the same number in the other line. However, in the long run this variation is probably not significant since the fluctuations in environment should be about the same in summation for one line as for the other.

From the nature of the method of selection, spores near the extremes of the range of variation were chosen, and accordingly the difference between the selected spore of the minus line, and that for the plus line in a given generation, was always large. The lengths of the selected spores for the experiments are shown in figure 8. The differences between the parent spores of the two groups does not increase significantly during the experiment, nor does it decrease. At the time the cultures were transferred to America the lengths of the parent spores decreased considerably but the deviations between them were unchanged. To what extent the decrease in spore length was due to change of culture medium, and to what extent due to change of climate, is unknown.

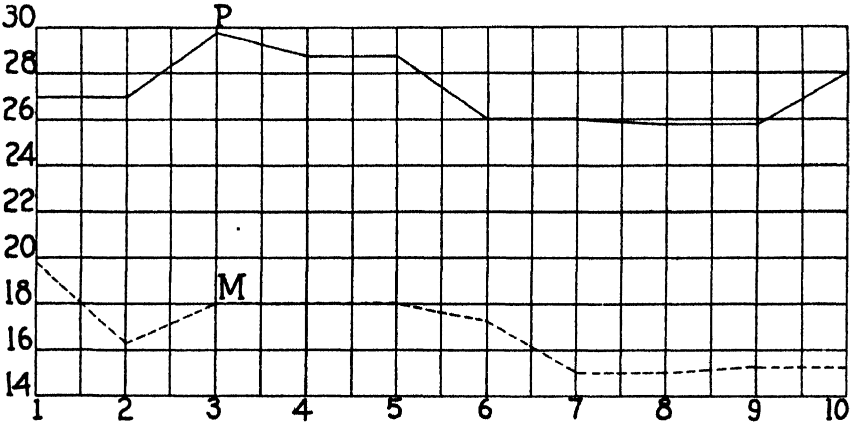

FIGURE 8.—Graphs of the lengths of individual spores selected as parents of the plus and minus line of experiment 3. P, the plus selections; M, the minus selections. The ordinates are lengths in μ; the abscissae, the successive generations.

The mean spore lengths of the generations of this experiment are shown in table 8. The removal of the cultures to America resulted in a decrease in mean spore length which is clearly shown in figure 9. The plus and minus lines did not parallel each other so closely as in experiments 1 and 2. This is to be expected because the selections in the two lines were not usually made at the same time. The two lines were consequently not in harmony in regard to the conditions under which they were developed and their fluctuations were not parallel. In five of the ten generations the minus line has a greater spore length than the plus-selected one. That this result is due to selection is unthinkable. It serves as a warning that small changes, apparently in the direction of a selection effect, should not too readily be considered as due to selection, though they have usually been so interpreted.

The means of means of all the cultures in the two lines when compared are found not to be significantly different. The upward and downward swings have had the same total effect in one line as in the other though the different lines have scarcely paralleled each other at all during these swings. Experiment 3, then, entirely substantiates the results gained in experiments 1 and 2, and shows that the selection of visible characters within a pure line is no more effective in producing distinct groups than selection on the basis of progeny.

In the course of experiment 3 an aberrant form appeared, which seems to be a mutation. This arose in the plus line in the seventeenth generation. In regard to length of spores and of spore appendages it is identical with strain 17 from which it arose. However, it has vegetative characters quite distinct from those of strain 17 and this makes it appear very different to the naked eye. Strain 17 produces a very small amount of mycelium on the surface of the agar substratum, which becomes almost completely covered with a black, slimy mass of spores. The new form produces a thick felt of mycelium all over the surface of the agar, and sporulation is much more tardy than in strain 17. The spores are produced in small black masses which are rather sparsely scattered over the surface of the

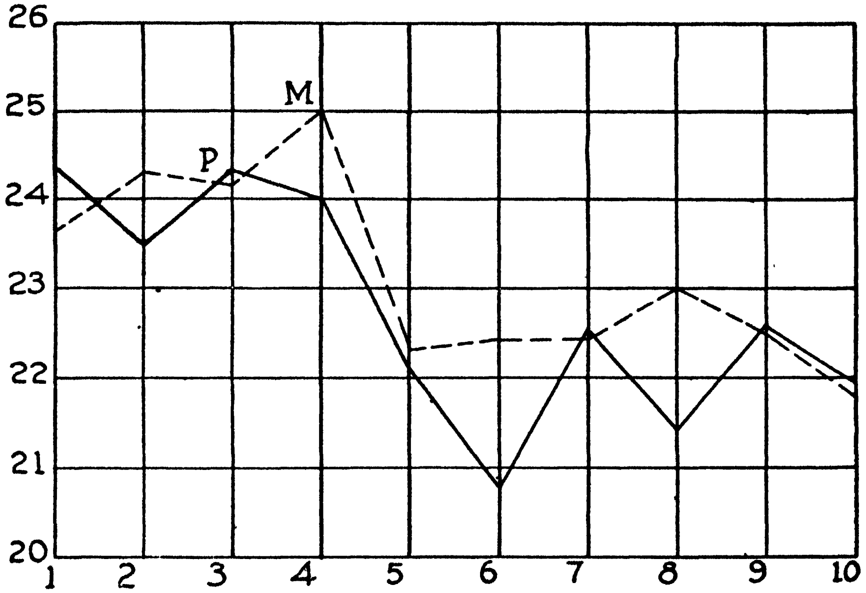

FIGURE 9.—Graphs of the spore lengths of cultures grown in experiment 3. P shows the cultures of the plus line; M those of the minus line. The ordinates are lengths in μ; the abscissae, the generations of the experiment.

mycelium. Thus far the form has remained distinct for two generations, and will be studied further in the near future. If it continues to show distinctive characters it will have to be considered as a mutation, though one which affected fewer characters than the one encountered in experiment 1. More detailed statements concerning both these mutations will be presented in another publication.

TABLE 8

Mean spore lengths of cultures grown in experiment 3. One culture was grown in each line in each generation. Each mean is based on 100 measurements. Measurements in μ.

GENERATIONS	PLUS SELECTIONS	MINUS SELECTIONS
1	24.37	23.65
2	23.53	24.30
3	24.34	24.16
4	24.01	25.04
5	22.09	22.30
6	20.78	22.40
7	22.56	22.46
8	21.43	22.98
9	22.58	22.51
10	21.97	21.76
Means of generation means	22.766 ± .251	23.16 ± .212
Standard deviations	1.18 ± .17	1.01 ± .15

Difference between plus-selected line and minus-selected line = 0.390 ± 0 328μ.

Experiment 4

It was originally intended that this experiment should be carried on for an extended period of time. In order to lessen the labor required, some changes were made in the method which had been used in experiment 3. The spores were selected and grown in the same manner as in experiment 3 but the cultures of each generation were not measured. Instead it was planned to measure them only from time to time to determine whether or not the plus and minus lines were diverging one from another. Unfortunately only a few selections had been made before the writer left Sumatra. The strain used, No. 5, which was isolated from a leaf spot of cocoanut palm and had been successfully grown for nine generations in culture with the results shown in figures 1 and 2, and in table 9, did not thrive well in Michigan, even when kept in a constant-temperature room, so after six selections the experiment was discontinued. While the experiment included fewer selections than is desirable, a large number, perhaps the majority, of selection experiments have been carried no farther, and no apology need be made for presenting the results. These are shown in table 10 and the measurements of the selected parent spores are presented in figure 10.

The spores selected, on the basis of spore length, as parents of the plus and the minus lines, were significantly different from each other in every generation, so that there can be no doubt that real selections were made.

TABLE 9

Mean spore lengths of cultures of strain 5, grown prior to the initiation of experiment 4. Measurements in μ.

GENERATIONS	SPORE LENGTHS	RANGE OF MEASUREMENTS
1	24.4	20–29
2	21.1	17–26
3	22.3	17–24
4	20.2	17–24
5	22.2	19–26
6	22.4	19–29
7	20.6	17–24
8	23.2	19–29
9	21.9	18–25
10	21.5	17–27
Mean	21.98	

The cultures were removed from Sumatra after three minus selections and two plus selections had been made. The fourth, fifth, and sixth selections of the plus line show no decrease in spore size following this change of climate, since they are all at least equal in size to the third selection, and the sixth selected spore equals the size of the first and second parent spores of the line. In the minus line, however, the third, fourth, and fifth selections show a decided decrease in spore size. The sixth selected spore is equal in size to the second, but it should be noted that another chosen spore of precisely the same size as the fifth selected spore failed to grow. It is rather uncertain whether any of these changes may be attributed to climatic changes.

Only the cultures of the fifth and sixth generations were measured. In the fifth generation the culture of the plus line had a greater mean spore length than that of the minus line, but in the next generation the result was reversed, and the culture of the minus line had the higher mean. The summation of the two generations shows that the two lines are not distinct, the difference between them being barely larger than its probable error. The result of this experiment is the same as that for the three preceding experiments, namely, that selection has no appreciable result.

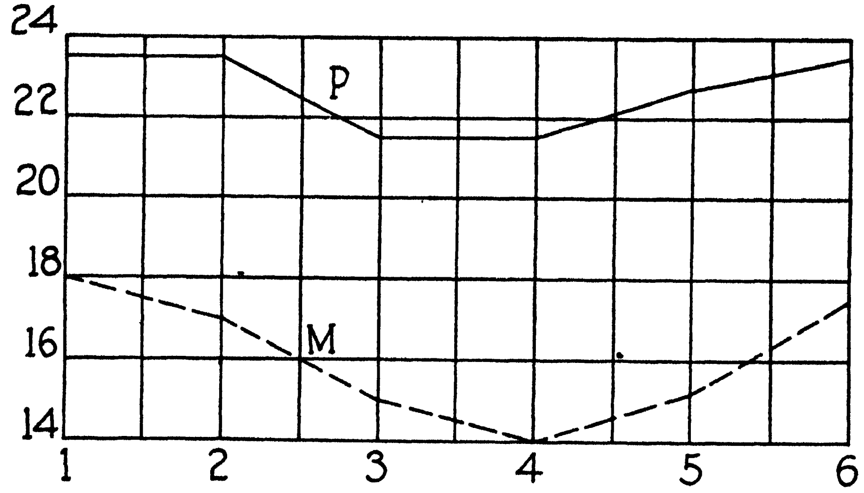

FIGURE 10.—Graphs showing the lengths of the individual spores selected as parents of the plus and minus lines of experiment 4. P indicates the plus selections; M, the minus selections. Lengths in μ are given as ordinates; the successive generations as abscissae.

The evidence gained from two experiments, in which visible characters were selected, is in entire agreement with that secured from the more extensive experiments in selection according to progeny, and all the experiments, whatever the method and strain used, consistently show that selection within pure lines of Pestalozzia is entirely ineffective, but that rarely mutations occur which are significantly different from the parent line.

DISCUSSION

JENNINGS (1916, 1920) considers that if the experiments which have been supposed to demonstrate the negative result of selection had dealt with characters which were less likely to be influenced by degree of maturity and environmental influences, had been more carefully conducted, and had involved a larger number of selections, they would likely have shown a positive result. The Pestalozzia investigation has greater evidential value. It was carried on for 10, 25, 10, and 6 generations, respectively, in the several experiments. The characters studied were such as to admit of certainty as to maturity of the observed individuals, and they were not more subject to environmental influence than those considered in investigations which have seemed to give evidence of an effect of selection. In certain respects, which have already been pointed out, the different generations have been more fully known, and a larger number of variates have been considered, than in any other investigation yet reported. Nevertheless, the different experiments consistently fail to show any effect of selection. The evidence secured in this study then is a direct confirmation of the results of JOHANNSEN and the other early workers on the pure-line problem. In attempting an explanation of the discrepancy between these results and those which contradict them, it is necessary to attempt to evaluate the experiments that have shown a positive selection effect.

TABLE 10

Means of generations measured in experiment 4. Measurements in μ.

GENERATION	PLUS-SELECTED LINE	MINUS-SELECTED LINE
5	21.142±.110	20.802±.117
6	20 428±.114	21.500±.112
Means	20 785± 241	21.151±.236

Difference between plus-selected line and minus-selected line=0.366±0.337μ.

The results of STOCKING (1915), while sufficiently striking and credible, can be given little weight in a general consideration since the abnormal characters dealt with so strongly suggest a pathological condition of the organism. To be given weight in a discussion of selection as a factor in evolution, the supposed modification produced by selection must affect a normal character in a direction shown by existing forms in nature to have been actually traversed by these other forms in the course of their evolution.

MIDDLETON (1915) found selection for fission rate in Stylonychia effective to a degree which must have been surprising, even to believers in the possibility of modification by selection. The great readiness with which organisms could be altered in MIDDLETON'S experiments indicates that the response of Stylonychia to selection is to some degree exceptional. ROOT (1918) suggests that "the inheritance of variations in fission rate in Stylonychia might be due to the accumulation of waste products in the cytoplasm of the slowly-dividing, large-sized group." The fact that MAST (1917) secured two lines with different fission rates in *Didynium nasutum* without selection is suggestive that mutation may offer an alternate explanation of MIDDLETON'S results.

ROOT (1918) working on Centropyxis has secured data worthy of consideration. In one experiment, using mass selection, he found a decided effect of selection on the number of spines after only four selections. However, the number of individuals studied was very small,—88 in the high series and 77 in the low series. In another mass selection the results were exactly opposed to those of the first; that is, the parents with a low spine number produced offspring with more spines than those from parents with a large number of spines. The latter result obviously cannot be due to selection, though had it stood alone it might have been so interpreted. The two experiments show the danger of drawing conclusions from a small number of individuals and a few selections.

In a third experiment in which individuals were selected according to the character of their progeny no considerable effect was gained until the fourth selection. From the fourth selection a large effect was secured. One selected individual gave offspring all of which had a high spine number. This individual (5aIa of ROOT'S series), the offspring of which are responsible for a decided increase in the number of spines in the plus series, may have been a mutation. The number of spines in the low series did not appreciably decrease during the selection period. The whole experiment included only a small number of individuals, 56 in the plus series and 51 in the minus, and only four selections were made.

In the fourth experiment in which mass selection for number of spines was practiced, two populations were segregated which varied in respect to spine number. The difference between the two populations was small and fluctuated from generation to generation. In the second selection period the difference was less than in the preceding period. The number of individuals observed was larger than in the other experiments but still relatively small. It would have been interesting to see what the result of a continuation of the cultures would have been. As it is, one suspects the separation to have been only a temporary one, such as is shown for Pestalozzia in table 4 of this paper.

HEGNER (1919) carried on extensive selection experiments on Arcella and finds that it is possible to separate by selection within families groups which are distinct in regard to the selected character. In his principal experiment, however, the effects of selection on number of spines are by no means consistent. The first set of selections produced a higher number of spines in the group selected for low spine number than in the group selected for high spine number. It is unfortunate that at this point a change was made in the method of selection. The author attributes the effect secured in further selections to the use of a better criterion for the selection. The effect may have been due to independent chance fluctuations within the two groups and not to selection at all but the change in procedure prevents this being detected. It would have been very interesting to know whether or not such divergences would have occurred had the original method been continued. Five further selection periods showed an increase in spine number in the group selected for high spine number and a decrease in the group selected for low number. The difference between the high and the low series, however, does not show a steady increase, but fluctuates from one selection period to the next. When the divergence between the two groups was at a maximum, selection was stopped, so that we do not know whether the divergence would have decreased or not had selection been continued.

On the cessation of selection the divergence between the groups decreases very little for one period. In the next period the divergence falls to almost nothing while in the next following period it again rises to nearly half that secured at the end of the six selection periods. It is approximately equal to that found in the second selection period. In the second selection period the increase in divergence is attributed to selection. To what is it due in this non-selection period? It seems logical to assume that it is due to the same cause in both cases, probably to the independent chance fluctuations of the two groups.

Further selection for one period in the high line resulted in a slightly larger number of spines in the group selected for low spine number than in that selected for high spine number. That this is due to selection is, of course, impossible.

Three selection periods were effective in that they developed two divergent lines within the low line established by the early selection. Here again the divergence fluctuates from period to period. After selection ceases the divergence becomes greater than it was during the selections. This again indicates that the divergence may be totally unrelated to the selection. In experiment 1 of this paper may be found similar divergences, which were only temporary.

In another experiment HEGNER practiced selection for diameter and spine number for a period of nine days. During this time distinct high and low lines were produced. In a non-selection period of thirteen days following the selections the divergence between the high and low series continued to increase to a marked degree. Obviously this continued increase was not due to selection; on the contrary one would expect a lessened divergence between the high and low series following the cessation of selection. The increased divergence during the non-selection period was almost entirely due to increase in size and spine number in the high series. That this series later produced some very large forms, and that great difficulty was experienced in securing offspring from this group are facts that may be indicative of abnormality in the group. As in HEGNER'S other experiments, relatively few individuals were studied.

The extensive and painstaking studies of JENNINGS (1916) on Difflugia present what appears to be the least questionable evidence of the effectiveness of selection yet published. In one experiment he made selections for seven periods, each of which, except the first, included one generation. Selection was made for number of spines, individuals with from 1 to 3 spines being chosen as parents for a "low" set, and those with from 5 to 8 spines being retained as parents of a "high" set.

The selection was apparently effective and is so interpreted by JENNINGS. Two lines were formed by the selection which differ in mean spine length. However, a closer examination shows that the divergence of the two lines varied from generation to generation. In the second period the divergence is equal to nearly a whole spine (.95 spines), but in the third period the divergence falls to .03 spines, which means the lines are really identical statistically. From the third period on the divergence continually rises and falls but never again reaches so high a point as in the second period. If the divergence is due to selection why is the effect so great in the second period, and why do further selections result in a decreased divergence?

The individual measurements are not published, but if we compute the error of the difference between the means of the two groups, using the generation means, we find it to be .103. The difference between the means of the two groups is .22 ± .103 spines. The divergence is barely more than twice its probable error and so is not statistically significant.

During the selection periods the two groups showed fluctuations in regard to diameter of shell and length of spine which are similar to those already noted for number of spines. Table 11 shows the divergence of the two groups for each of the three characters.

It may be seen at a glance that the differences do not steadily increase with an increased number of selections but rise and fall as if by chance. The difference in spine number is greatest in the second period, that of shell diameter is greatest in the fourth period; and that for spine length is greatest in the fifth period, falling to a minus value in the sixth period. The mean for shell diameter is the only one which is equal to three times its probable error. Apparently the two lines are not demonstrably distinct.

In family 314 also, JENNINGS attempted to isolate lines distinct for spine number. Selection was continued for seven periods in the same way as within family 304. The means of the "high"-selected line and of the "low"-selected line were separately computed for each generation. The differences between the two lines varies from generation to generation as in family 304. It is noteworthy that the greatest divergence (1.24 spines) appears in the first selection period; the lowest deviation in the sixth period. The difference between the mean numbers of spines for the two groups is .42±.25 spines. Since the divergence is less than twice its probable error, we can only conclude that the lines are not distinct and that the apparent difference is due to the independent fluctuation of the two groups.

TABLE 11

Differences between "low" and "high" selected groups of family 303 of Difflugia.

SELECTION PERIODS	DIFFERENCES IN NUMBER OF SPINES	DIFFERENCES IN SHELL DIAMETER IN UNITS OF SCALE	DIFFERENCES IN LENGTH OF LONGEST SPINES IN SCALE UNITS
1	.41	.52	.48
2	.95	.63	.57
3	.03	1.16	.39
4	.35	2.88	.02
5	.25	.61	.67
6	.34	.95	.29
7	.60	.94	13
Mean	.22± 103	.93±.26	76±.299

In another and more extensive experiment, JENNINGS sought to isolate distinct lines by selection within family 326. Again spine number was the character selected, and selection was continued for twelve periods. For the first six periods selection was ineffective and both plus and minus divergences between the "low" and the "high" groups appeared.

As in one of HEGNER'S experiments, a change in the method of selection, made at this time, prevents us from knowing what would have resulted from further selections in which large numbers of individuals were used. Following the change in method two series distinct for spine number were isolated by selection during six periods. The difference between the means is 0.51 ± 0.109 spines or less than five times its probable error.

During the six selection periods following the change in the method of selection (periods 11 to 16) the divergence between the two groups did not constantly increase but fluctuated from period to period. In the eleventh period it was .52 spines, in the twelfth it fell to .49 spines, in the thirteenth it rose to .84, the highest point reached. In the fourteenth period the difference fell to .28 spines, in the fifteenth to .23 spines, which is probably not significant, and in the sixteenth or last period it again rose to .66 spines. If the divergence in period thirteen is due to selection, it is difficult to see why further selection for the same number of periods that caused this divergence, should cause a decrease in the difference of the two groups.

After selection was discontinued the two groups were kept under observation during five different periods which varied from eight to twenty-two days in length. The mean spine number of the individuals in the low-selected group was 5.19 while that of the high-selected group was 5.58. The error of the difference between the means of the two lines is 0.178 spines while the difference is only 0.39 spines, or slightly more than twice its probable error. The difference would not be considered significant if it were not for the fact that the variations in the various lines, though great, are insufficient to bridge the gap between oppositely selected lines. Is it a fair criticism that Difflugia is insufficiently plastic to show rapid changes from a certain condition of the shell after that condition is once attained? The results suggest that the shell itself plays a part in determining the character of the new individual formed by division of the old one. This shell effect may be regarded as superposed upon the other factors determining the form of the new shell. Such an effect might be expected upon physical grounds. Naked protoplasm protruded from a large orifice is under different surface conditions than if the orifice were smaller; the extruded protoplasm would be expected to assume a different form and size in the two cases, and the newly secreted shell would therefore be different. In other words, any accidental size modification might be maintained through several generations, not through any protoplasmic modification, but merely because the nature of the shell imposes certain physical conditions upon the development of the new individuals. A modification having once occurred in the shell through any cause, becomes comparable to an environmental factor which is maintained for several successive generations. The nature of new individuals is largely determined by the environment, and of the environmental factors the shell is one of the chief. If this suggestion has any validity, it follows that the two groups, while apparently different, were not significantly different, even while selection was being practiced.

If two lines are isolated within a strain and cultivated separately these lines will be independently subjected to the environmental influences which cause variation for the character studied. The lines will tend to coincide if they are subjected to these influences at the same time and to the same degree. This is the case with the strain of Pestalozzia studied in the author's experiment 2 and is shown in figure 7. It is apparent that there is a certain rhythm, either in the organism itself or in the environmental factors, which causes upward and downward swings of the organism in respect to the character under observation. Since great care was taken to have the two groups subject to identical conditions at all times the two groups follow one another very closely in these swings. In experiment 1 the swings follow each other less closely but with some exceptions there is a strong similarity of behavior in the two lines as is shown in figure 5. In MAST's work with Didynium, where two groups have been separated, apparently by a mutation, these groups fluctuate in the same direction at the same time, with remarkable unison.

If two lines are isolated and cultivated separately, but the offspring are produced in such a way that those of the two lines are subjected to the environmental factors causing variation for a given character, at a different time, or in a different degree, each of the lines will fluctuate in a different way, and this will cause divergence between the two. However if the lines are continued the fluctuations will compensate one another so that in the end the two lines will be found to give practically the same mean value for the character studied.

To the writer it appears that JENNINGS's results with Difflugia, ROOT's with Centropyxis, and HEGNER's with Arcella, are explained by the above assumption quite as well as are his own with Pestalozzia. If this be true they tend rather to demonstrate the ineffectiveness of selection within pure lines than the opposite.

It is warranted to express the belief that the "pure-line" hypothesis is still valid. The writer does not wish to express an opinion that ultimately the effectiveness of selection may not be shown, but he feels that the burden of proof lies with those who question the results of JOHANNSEN and his followers. At the present time incontestable evidence of an effect of selection is wanting. From the recent investigations made in this field it is obvious that the situation is vastly more complicated than was formerly supposed. The truth or falsity of the "pure-line" hypothesis will be finally determined by numerous investigations with various organisms, involving infinite care and toil. There is need of more investigations of the type which JENNINGS has made on Paramecium and Difflugia, but carried for longer periods, with organisms more favorable for study, if they may be found.

Nothing has been said in this paper, of the bearing of the "pure-line" hypothesis on theories of evolution. Almost every worker who has made careful biometric studies of highly variable groups has recorded the occurrence of mutations or "something like mutations." JENNINGS, ROOT, MAST, and HEGNER have all recorded them for the lower animals. BARBER (1907) noted the occurrence of mutation in yeasts; STEVENS (1920) has reported a number of mutations in Helminthosporium; and the writer has found mutations in Pestalozzia. It is unnecessary to catalogue the numerous cases of mutation recorded in various groups of higher plants and animals. Further careful study will doubtless reveal many more. When more is known of their occurrence, frequency, and reaction to environment, to other existing organisms, and to one another, may we not be able to conceive of evolution, even without cumulative growth of characters by the selection of fluctuating variations?

CONCLUSIONS

Selections according to progeny within pure strains of Pestalozzia were made for length of spores for ten generations, and for length of spore appendages for twenty-five generations, without result. Selections according to visible characters were made for spore length for ten generations in one experiment and for six generations in another, without establishing differences between the plus-selected and minus-selected lines. Whatever method and strain may be employed, selection is totally ineffective in establishing distinct lines within pure strains of *Pestalozzia Guepini.*

Mutations which give rise to lines significantly different from the parent lines, infrequently occur.

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APPENDIX

Tables of constants of experiments 1 and 2

TABLE 12

Means and standard deviations of spore lengths of cultures grown in experiment 1. The mean of each culture was calculated from at least 100 measurements. Values are given in units of the scale of the eye-piece micrometer used. 1 unit = 1.8 microns. Single spore culture of parent strain (No. 29). Mean = 13.80, σ = 1.58.

Generation 1 of experiment

CULTURE	MEAN	σ	CULTURE	MEAN	σ
1	14.46	1.55	11	14.16	1.43
2	13.90	1.76	12	13.72	1.82
3	13.61	1.85	13	13.64	1.43
4	13.97	1.63	14	13.62	1.54
5	13.51	1.79	15	14.18	1.84
6	14.02	1.90	16	13.58	1.67
7	13.71	1.80	17	13.85	1.87
8	13.57	1.71	18	13.79	1.82
9	13.95	1.47	19	13.95	1.65
10	13.71	1.71	20	13.89	1.52

Mean of generation 13.84.

Generation 2

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	14.03	1.41	14.23	1.66	13.40	1.10
2	14.29	1.44	13.99	1.38		
3	14.94	1.52	14.19	1.39		
4	13.95	1.43	14.00	1.29		
5	14.33	1.49	13.82	1.39		
6	14.35	1.52	14.00	1.27		
7	14.94	1.55	14.31	1.43		
8	13.43	1.33	14.12	1.52		
9	13.72	1.54	14.17	1.62		
10	13.94	1.93	14.15	1.33		
Generation means	14.19		14.10		13.40	

TABLE 12 (continued)

Generation 3

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	14.46	1.26	13.55	1.24	14.77	1.38
2	13.88	1.28	13.92	1.22		
3	14.19	1.21	13.60	1.38		
4	14.11	1.09	13.61	1.29		
5	14.34	1.25	13.60	0.96		
6	13.75	1.18	13.54	1.18		
7	13.75	1.16	13.79	1.33		
8	14.22	1.33	13.77	1.21		
9	13.74	1.07	13.59	1.33		
10	13.88	1.15	13.70	1.31		
Generation means	14.03		13.67		14.77	

Generation 4

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.50	1.17	13.27	1.13	13.55	1.14
2	13.62	1.22	13.68	1.24		
3	13.87	1.04	13.63	1.32		
4	13.11	1.12	13.79	1.13		
5	13.37	1.11	13.32	0.99		
6	13.93	1.33	13.09	1.29		
7	13.78	1.12	13.64	1.07		
8	13.53	1.37	13.44	1.29		
9	13.27	1.01	13.55	0.95		
10	13.59	1.21	13.52	1.07		
Generation means	13.56		13.49		13.55	

TABLE 12 (continued)

Generation 5

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.92	1.17	13.93	1.12	14 03	1.32
2	13.55	1.04	14.19	1.26		
3	14.07	1.31	13 43	1.29		
4	13.60	1.24	13.75	1.13		
5	13.77	1.02	13.41	1.21		
6	13.88	1.10	13.71	1.42		
7	13.40	1.19	13.81	1.10		
8	13.47	1.37	14.14	1.27		
9	14.05	1.02	13.53	1.12		
10	13.63	1.10	14.04	1.19		
Generation means	13.73		13.79		14.03	

Generation 6

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.73	1.17	13.75	1.18	13.31	1.12
2	13.74	1.18	13.65	1.19		
3	13.91	1.12	13.75	1.33		
4	14.14	1.01	13.60	0.88		
5	13.62	1.05	13.23	1.08		
6	13.65	1.00	13.87	1.06		
7	13.64	1.11	13.68	1.18		
8	13.69	1.10	14.10	1.13		
9	13.84	1.20	13.66	1.36		
10	13.50	1.15	13.91	1.24		
Generation means	13.75		13.72		13.31	

TABLE 12 (continued)

Generation 7

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.84	1.28			13.26	1.06
2	13.76	1.21				
3	13.59	1.13	14.17	1.40		
4	13.78	1.09				
5	13.33	1.33				
6	14.08	0.98				
7	13.86	1.12	13.76	1.33		
8	13.45	0.97	13.67	1.37		
9	13.87	1.22	13.17	1.66		
10	13.75	1.22				
Generation means	13.73		13.69		13.26	

Generation 8

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.46	0.95			13.47	0.98
2	13.65	1.11				
3	13.79	1.17	13.03	1.01		
4	13.93	1.06	13.79	1.05		
5	13.89	1.07	13.49	0.98		
6	13.18	1.11	13.60	1.15		
7	13.42	1.05	13.59	1.13		
8	13.80	1.14	13.17	1.08		
9	13.47	1.08				
10	13.49	1.15				
Generation means	13.61		13.45		13.47	

TABLE 12 (continued)

Generation 9

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.46	1.23	13.43	1.07	13.29	1.03
2	13.50	1.17	13.56	1.21		
3	13.28	1.08	13.92	1.03		
4	13.71	1.13	13.84	1.02		
5	13.26	1.06	13.57	0.99		
6	13.53	1.09	13.21	1.34		
7	13.55	1.31	13.47	1.11		
8	13.45	1.04	13.68	1.05		
9	13.38	1.17	13.25	0.98		
10	13.31	1.08	13.75	1.08		
Generation means	13.44		13.57		13.29	

Generation 10

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	13.45	1.22	13.05	1.03	13.35	1.03
2	13.31	1.15	13.13	1.38		
3	13.89	1.33	13.14	1.00		
4	13.03	0.95	12.96	1.02		
5	13.71	0.94	13.27	0.91		
6	13.86	0.96	13.26	0.88		
7	13.67	0.99	13.19	1.18		
8			13.12	1.08		
9	13.57	1.11	12.92	1.17		
10	13.54	1.04	13.44	1.07		
Generation means	13.56		13.15		13.35	

Means of all cultures in experiment 1

PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
13.736±0.0311 σ=0.435±0 0219	13.679±0.0226 σ=0.331±0.0159	13.640±0.070 σ=0.326±0.0492

Difference between plus selections and minus selections = 0.057 ± 0.038
Difference between plus selections and intermediate group = 0.10 ± 0.058
Difference between minus selections and intermediate group = 0.039 ± 0.054.

TABLE 13

Means and standard deviations of appendage lengths of cultures studied in experiment 2. The mean of each culture was calculated from 100 or more measurements. Values are given in units of micrometer scale: 1 unit = 1.8 microns. Single-spore culture of parent strain (No. 29). Mean = 12.42, $\sigma = 2.08$.

Generation 1 of experiment

CULTURE	MEAN	σ	CULTURE	MEAN	σ
1	12.67	2.20	11	13.62	2.21
2	12.50	2.22	12	13.52	3.07
3	13.58	2.30	13	13.69	2.96
4	13.57	2.40	14	13.48	2.55
5	13.85	2.43	15	12.48	2.54
6	13.46	2.26	16	14.01	2.52
7	13.64	2.52	17	12.01	2.81
8	13.10	2.30	18	13.08	2.42
9	13.66	2.34	19	13.74	2.72
10	13.81	2.29	20	12.46	2.33

Mean of generation 13.30

Generation 2

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.70	2.00	11.59	2.17	12.80	2.28
2	12.16	1.98	11.42	2.24		
3	12.09	1.95	11.60	2.31		
4	12.03	1.88	11.44	2.07		
5	11.92	2.26	11.40	2.06		
6	11.44	2.19	10.63	1.91		
7	11.49	2.03	11.36	2.22		
8	11.92	2.71	11.60	2.13		
9	11.14	2.16	11.68	2.29		
10	11.97	1.98	11.42	2.03		
Generation means	11.79		11.41		12.80	

TABLE 13 (continued)

Generation 3

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.05	1.77	10.31	2.34	11 55	2.33
2	9.99	2.20	10.41	1.67		
3	9.84	2.07	10.44	2.20		
4	11.29	2.17	11.01	2.09		
5	10.64	2.16	10.78	2.19		
6	10.85	2.08	10.17	1.60		
7	10.48	2.12	10.01	2.04		
8	10.53	2.07	10.11	1.76		
9	10.75	1.96	10.49	2.27		
10	11.52	1.99	10.41	2.10		
Generation means	10.59		10.41		11.55	

Generation 4

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.60	1.88	12.42	2.08	11.60	2.08
2	9.99	2.16	11.69	2.60		
3	11.04	1.83	10.97	1.98		
4	10.61	2.18	11.32	1.83		
5	10.18	1.94	11.78	2.36		
6	11.48	2.18	11.23	2.43		
7	12.14	2.17	10.99	2.03		
8	11.40	2.20	11.72	2.27		
9	11.18	1.77	11.82	2.36		
10	10.55	1.93	11.22	2.17		
Generation means	10.92		11.52		11.60	

TABLE 13 (continued)

Generation 5

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.82	2.46	11.93	1.96	11.55	2.43
2	12.22	2 08	10 95	2 44		
3	11.60	1.98	10.51	2.31		
4	12.42	2.38	11.13	2.67		
5	11.64	2.17	10 54	1.96		
6	12.33	2 12	11 52	2.14		
7	12.39	2 22	11.17	2.35		
8	12 19	2 30	10.26	2.46		
9	12.04	2 43	11.15	2.28		
10	12.33	2.63	10.45	2.37		
Generation means	12 10		10 96		11.55	

Generation 6

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.96	2.57	11.79	2.36	12.57	2.34
2	11.92	2 36	11.47	2.31		
3	11.91	2.13	11.39	2.30		
4	11.04	2.31	12.40	2.21		
5	11 60	2.16	12 00	2.37		
6	11.50	1 90	11.08	2.18		
7	11.80	2.60	11.88	2.41		
8	11.58	1.86	11.82	2.47		
9	11.19	2.61	11.20	2.31		
10	11.93	2.61	12 02	2.84		
Generation means	11.54		11.71		12.57	

TABLE 13 (continued)

Generation 7

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	12.11	2.16	11.86	2.34	12.45	2.30
2	12.34	1.98				
3	12.08	2.25	12.29	2.58		
4	12.10	2.27	12.36	2.22		
5			12.49	2.74		
6	12.16	2.35	12.14	2.15		
7	11.87	2.31	11 94	2.25		
8	12.38	1.86	12.35	2.44		
9	12.25	2.13	12.25	2.10		
10	12.30	1.65	11.82	2.20		
Generation means	12.18		12 16		12 45	

Generation 8

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.58	1.99	11.84	2.09	11.65	2.18
2	11.72	1.96	11.91	2.20		
3	12.47	1.94	10.68	1.74		
4	11.41	2.26	11.92	2.05		
5	11.42	2.12	12.08	2.14		
6	11.71	2.17	12.03	2.09		
7	11.51	2.22	12.34	2.02		
8	11.39	2.24	11.23	2.19		
9	11.95	2.15	12.04	2.33		
10	10.81	2.18	11.87	2.30		
Generation means	11.60		11.79		11.65	

TABLE 13 (continued)

Generation 9

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.45	1.98	10.40	1.92	10.46	2.13
2	10.88	2.16	10.83	1.94		
3	10.66	2.12	9.47	2.04		
4	10.21	2.03	9.37	1.86		
5	10.27	1.94	9.75	1.84		
6	10.05	2.00	10.30	2.06		
7	10.59	2.23	9.85	2.15		
8	9.70	2.02	10.13	2.17		
9	9.95	1.74	10.32	2.02		
10	10.63	2.16	10.81	1.93		
Generation means	10.34		10.12		10.46	

Generation 10

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	9.07–	1.86	10.19	2.07	9.47	1.55
2	9.49	1.79	9.64	2.20		
3	9.27	1.98	10.03	2.14		
4	10.20	1.80	9.82	1.84		
5	9.13	1.69	10.00	1.60		
6	9.88	2.07	9.87	2.03		
7	9.54	1.68	9.98	1.93		
8	10.22	1.92	9.79	1.69		
9			9.84	1.99		
10	9.88	1.94	10.18	1.97		
Generation means	9.63		9.93		9.47	

TABLE 13 (continued)

Generation 11

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.82	2.09			12.60	1.93
2	12.30	2.25				
3	12.07	2.20				
4	11.32	2.14				
5	11.33	2.17				
6	12.23	2.19				
7	11.99	2.07				
8	11.75	2.10				
9	11.46	2.02				
10	10.95	1.83	11.87	2.13		
Generation means	11.62		11.87		12.60	

Generation 12

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.22	2.09	11.40	2.08	11.19	1.95
2	11.07	1.96	10.33	2.11		
3						
4	10.43	2.05	10.94	2.27		
5	10.76	2.12	10.24	1.78		
6	10.63	2.09	10.92	2.22		
7						
8	10.77	2.17	10.91	2.14		
9						
10	10.69	1.94	10.22	2.01		
Generation means	10.79		10.71		11.19	

TABLE 13 (continued)

Generation 13

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.53	2.04	9.77	1.76	10.37	2.05
2	10.07	1.87	10 49	2.16		
3	11.22	2.08	10.75	2.38		
4	10.61	2.33	10.65	1.69		
5	9.76	2.09	10.72	2.32		
6	10.52	2.20	10.04	1 99		
7	10.94	2.33	10.87	2 18		
8	10 66	1.93	10 94	2.19		
9	10.35	2.31	10.19	2.26		
10	10.83	2.25	9.63	2.32		
Generation means	10 55		10 41		10.37	

Generation 14

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.48	1.92	10.58	2 15	10.33	2.10
2	10 47	1.76	12.16	2.17		
3	10.64	1.82				
4	10 74	1.83	10.33	2.13		
5	11 98	2.55	14.85*	2.70		
6	12.52	2.33				
7	11.71	2.11	11.24	2.35		
8	12.29	2.16				
9	11.29	2.10	12.30	1.98		
10	11.68	2.22	11.76	2.22		
Generation means	11.48		11.90		10.33	

*Mutant.

TABLE 13 (continued)

Generation 15

CULTURES	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.67	2.11	11.78	2.08	11.80	2.46
2	11.78	2.07	11.47	1.76		
3	11.90	1.87	12.12	2.11		
4	11.34	2.34	11.65	1.91		
5	11.75	2.04	11.36	2.02		
6	11.33	2.33	11.07	1.79		
7	11.35	2.28	11.20	1.98		
8	12.50	2.24	11.71	2.06		
9	11.27	2.21	11.77	2 04		
10	11.22	1.85	10.47	2.19		
Generation means	11.61		11.46		11.80	

Generation 16

CULTURES	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.37	2.08	10.86	2.05	11.00	2.45
2	11.52	2.10	10.41	2.09		
3	10.50	2.07	10.79	2.21		
4	11.57	2.01	10.82	2.26		
5	11.27	2.07	10.55	1.97		
6	11.49	1.98				
7	11.17	2.33	11.25	1.96		
8	11.76	2.25	10.73	1.73		
9	12.09	1.78	10.22	1.65		
10	11.77	2.04				
Generation means	11.45		10.70		11.00	

TABLE 13 (continued)

Generation 17

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	10.70	1.98	9.97	1.63	10.34	1.81
2	9.83	1.82	10.60	1.93		
3	10.36	1.77	9.92	1.87		
4	10.46	2.01	10.09	1.75		
5	10.61	1.92	9.88	1.80		
6	10.32	2.16	10.37	1.87		
7	10.17	1.72	10.01	1.73		
8	10.87	1.67	10.17	2.00		
9	10.01	1.83	10.60	2.21		
10	9.80	1.96	10.29	1.86		
Generation means	10.31		10.19		10.34	

Generation 18

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	9.56	1.97			10.80	1.95
2	9.94	2.43	10.66	2.02		
3	10.47	1.82	9.92	1.54		
4	10.36	2.01				
5	10.00	1.78				
6	9.72	2.08	10.45	1.74		
7			10.46	1.93		
8						
9	10.02	2.16	10.26	1.74		
10	9.85	2.04	10.52	1.79		
Generation means	9.99		10.39		10.80	

TABLE 13 (continued)

Generation 19

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1			11.60	1.74	11.37	1.89
2	11.49	2.08	11.41	1.97		
3	11.12	2.02	11.22	1.97		
4	11.14	2.16	10.85	1.99		
5	11.12	2.18	10.72	1.69		
6	11.49	2.07	11.71	2.11		
7	11.69	1.96	11.91	2.28		
8	11.61	1.98	12.30	2.04		
9	11.44	1.99	12.15	1.98		
10			11.79	2.18		
Generation means	11.49		11.57		11.37	

Generation 20

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.59	2.05	11.35	1.91	10.79	1.81
2	10.89	2.05	11.11	1.86		
3	11.00	2.11	11.05	1.93		
4	10.55	1.93	11.14	1.94		
5	10.69	2.00	10.68	2.18		
6	11.22	1.90	11.41	1.96		
7	10.68	1.91	10.88	1.78		
8	10.98	2.14	10.95	1.81		
9	10.53	2.02	10.02	2.03		
10	10.86	2.07	10.49	2.10		
Generation means	10.90		10.90		10.79	

TABLE 13 (continued)

Generation 21

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.70	2.26	11.43	2.01	10.36	2.01
2	11.47	1.96	11.01	1.87		
3	10.49	1.92	9.64	1.70		
4	10.74	1.69				
5	10.80	2.09	10.86	2.00		
6			11.37	1.94		
7	10.78	1.98	10.88	2.10		
8	8.99	1.87	11.44	1.99		
9	11.95	2.09	10.46	2.02		
10	12.21	2.04	10.59	1.84		
Generation means	11.13		10.85		10.36	

Generation 22

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.76	2.07	11.96	2.00	10.58	1.94
2	11.22	2.10	10.85	1.99		
3	11.14	2.00	11.39	2.33		
4	11.06	1.74	10.75	2.26		
5	10.66	1.74	11.21	1.74		
6	11.11	1.95	11.76	2.32		
7	11.37	2.15	12.16	2.20		
8	11.42	2.14	11.56	1.67		
9	11.38	2.14	11.30	1.87		
10	11.04	1.83	11.80	2.30		
Generation means	11.22		11.47		10.58	

TABLE 13 (continued)

Generation 23

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.37	2.02	10.89	1.95	12.13	2.28
2	10.80	1.92	11.29	1.84		
3	11.29	1.77	10.96–	1.80		
4	11.50	1.80	10.82	1.81		
5	10.94	1.95	11.50	1.96		
6	11.30	1.83	11.53	1.77		
7			11.44	2.05		
8	10.98	1.90	11.28	1.86		
9	10.98	1.91	11.56	1.88		
10	10.87	1.82	11.46	1.99		
Generation means	11.11		11.27		12.13	

Generation 24

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1	11.48	2.26	11.48	1.92	11.70	2.52
2	11.56	2.23	10.88	2.25		
3			10.40	1.89		
4			11.35	1.98		
5			10.63	2.02		
6			11.26	2.22		
7			11.11	2.00		
8			11.22	1.93		
9			11.37	2.14		
10			11.46	2.17		
Generation means	11.52		11.12		11.70	

TABLE 13 (continued)

Generation 25

CULTURE	PLUS SELECTIONS		MINUS SELECTIONS		INTERMEDIATE GROUP	
	Mean	σ	Mean	σ	Mean	σ
1			10.33	1.62	10.90	1.91
2			10.45	2.00		
3			11.45	2.23		
4			10.44	1.91		
5	10.58	2.17	10.46	1.96		
6			10.91	1.87		
7			10.72	1.85		
8	10.87	2.31				
9						
10						
Generation means	10.73		10.68		10.90	

Means of all cultures in experiment 2

PLUS SELECTIONS	MINUS SELECTIONS	INTERMEDIATE GROUP
11.330±0.469 σ=1.06±0.033	11.235±0.043 σ=0.97±0.030	11.39±0.126 σ=0.034±0.089

Difference between plus and minus selections = 0.095 ± 0.064
Difference between plus selection and intermediate group = 0.060 ± 0.134
Difference between minus selections and intermediate group = 0.155 ± 0.133.

GENETIC INTERRELATIONS OF TWO ANDROMONOECIOUS TYPES OF MAIZE, DWARF AND ANTHER EAR[1]

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INTRODUCTION

Attention was called by MONTGOMERY (1906) to the occasional appearance of perfect flowers in the staminate inflorescence of maize and similar cases were reported by KEMPTON (1913). MONTGOMERY (1911) described with illustrations a true-breeding type of semi-dwarf dent maize, the ears of which were perfect-flowered. Perfect-flowered maize was described and illustrated also by BLARINGHEM (1908, pp. 180-183). EAST and HAYES (1911, pp. 13, 14) noted and illustrated a perfect-flowered sweet corn. WEATHERWAX (1916, 1917) showed that typically pistillate flowers of maize exhibit in microscopic sections the rudiments of stamens and that staminate flowers show rudiments of pistils.

The senior author of this paper (EMERSON 1911, pp. 82-87; 1912) described and illustrated dwarf and semi-dwarf andromonoecious maize types and showed that they are inherited as simple recessives to monoecious forms of normal height. Later work has shown that two genetically distinct types, now known as "dwarf" and "anther ear," were confused in these accounts. It was noted in one of them (EMERSON 1911) that the andromonoecious forms of unrelated stocks differed much in height, but the differences were thought to be due to growth factors modifying the height of dwarfs as multiple factors are assumed to differentiate normal races of diverse heights.

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[1] Paper No. 90, Department of Plant Breeding, CORNELL UNIVERSITY, Ithaca, New York.

CHARACTERISTICS OF THE DWARF AND ANTHER-EAR TYPES

Perhaps the most nearly unique feature of both dwarf and anther ear is the presence of stamens throughout their ears (figures 1 and 2). Sometimes one flower of the spikelet has three well developed stamens and the other flower more or less rudimentary ones but in other cases both flowers have fully developed stamens. Since the terminal inflorescences of dwarf and anther-ear plants have staminate flowers only, the types are andromonoecious. Exceptions to this condition, that is, dwarf and anther-ear plants without ear stamens or with rudimentary ones only, have been observed and are discussed in a later section of this account.

The ears of both dwarf and anther ear generally end in thick unbranched tassel-like spikes having staminate flowers only. Anther-ear plants in which the ear is represented by only a few seeds at the base of these club-like staminate-flowered spikes are common, and one plant was found that lacked even these few seeds and that was therefore wholly staminate-flowered. Some stocks of both dwarf and anther ear, on the contrary, have no tassel-like appendages to their ears. In any case the ears are short and relatively thick (figure 1).

The tassels of dwarf plants are compact and little branched, often quite unbranched. The smaller forms of the anther-ear type have tassels that are very similar to those of dwarfs in both compactness and branching while the taller forms usually have tassels that are more slender and more branched than those of dwarfs but rarely so much so as those of normal plants (figures 3 and 4). Our early cultures of dwarfs shed little or no pollen, due apparently to failure of the glumes to open normally. Pollen collected from anthers that had been removed from the tassel proved to be functional. Two dwarf plants found in a culture grown in 1913 shed pollen abundantly. By selection in later cultures of dwarfs descended mostly from these two plants, dwarf strains have been obtained that shed pollen fairly well.

The dwarf type is further characterized by very short culms, ordinarily not more than one-fourth the length of the culms of related normal plants (figure 3). Anther-ear plants also are usually considerably shorter than normals. There are, however, marked differences between different stocks of anther ear with respect to height, some being definitely semi-dwarf and some only slightly shorter than related normal plants (figures 3 and 4).

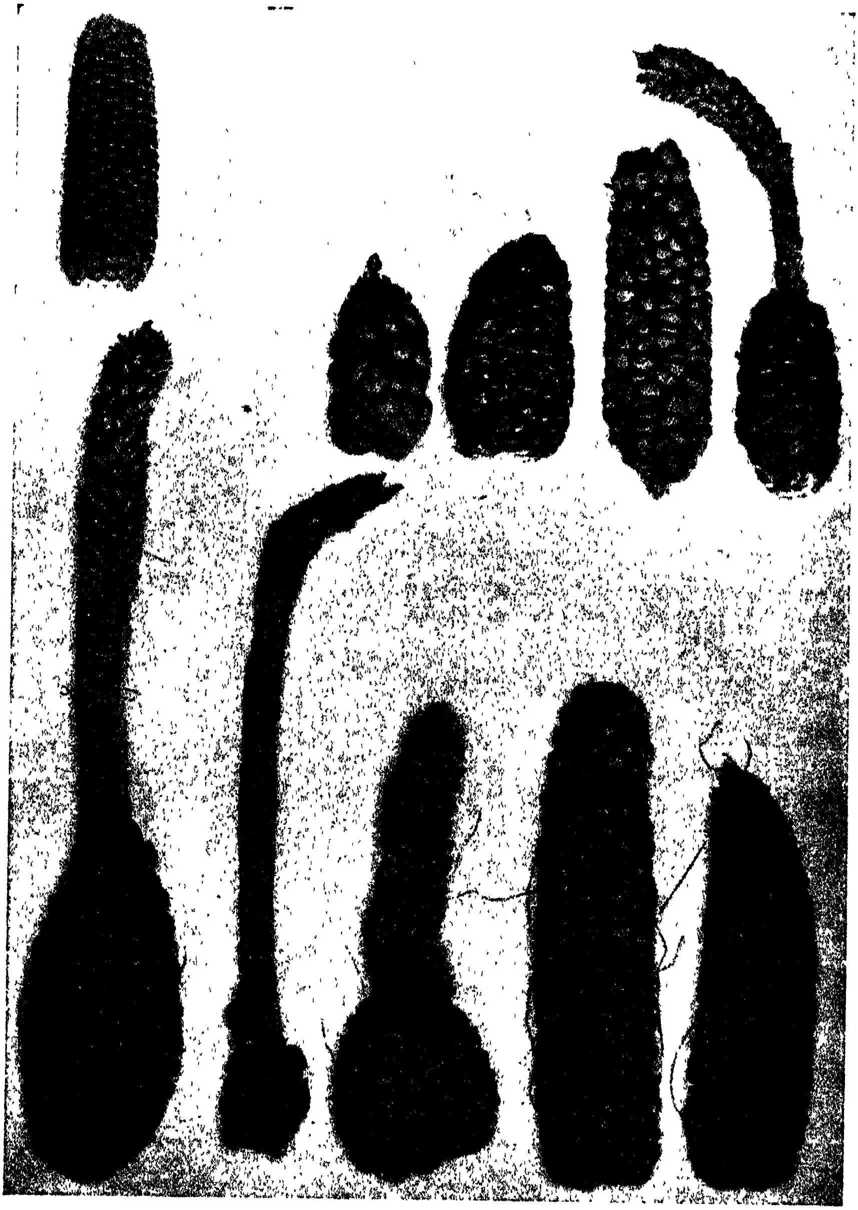

FIGURE 1.—Representative ear types. Below, five anther ear; above, right, four dwarf; above, left, one Tom Thumb.

The possible significance of this variation in height is considered in a later section of this paper. The differences in height are apparent even in the seedling stage (figure 5), though the taller forms of anther-ear seedlings are not always greatly different from normals.

The leaves of dwarf plants are characteristically short and broad. The first seedling leaves are not only shorter but actually broader than those of normal plants and the leaves of mature dwarf plants, though very short, are often quite as broad as those of normal plants. The leaves of most anther-ear plants also are relatively short and broad, being in this respect as a rule intermediate between those of dwarf and those of normal plants. The leaves of the taller anther-ear stocks are often not very different from those of normal plants.

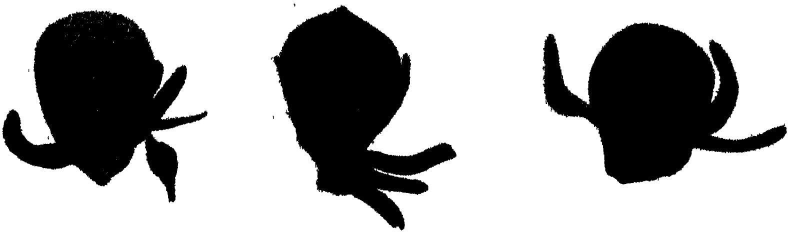

Figure 2—Seeds of anther ear and dwarf with stamens attached.

The low stature of dwarf and of anther-ear plants is due to shorter internodes than those of normal plants. In number of internodes these types are not characteristically different. In contrast to this condition of dwarf and anther ear, the low stature of such varieties as Tom Thumb pop is due to the small number of internodes, not merely to short ones (figure 4). Tom Thumb is normal not only in lacking ear stamens (figure 1) but in the relative length, breadth and thickness of its parts. Its ears, tassels, culms and leaves are like those of normal tall plants except that they are proportionately smaller in all dimensions. Dwarf and anther ear, on the other hand, are dwarfed in all longitudinal dimensions but not correspondingly so in diameter of parts.

A type of extreme dwarfness, known as "dwarf anther ear," the double recessive of dwarf and anther ear, as its name indicates, has appeared in certain crosses discussed in a later part of this account (figures 3, 5 and 6). This type is much smaller even than the type called "dwarf." It is characterized by an excessive shortening of all longitudinal dimensions. The length of culm, including the tassel, is often not over 12 centimeters. Its largest leaves are not far from 12 centimeters long and 6 centimeters

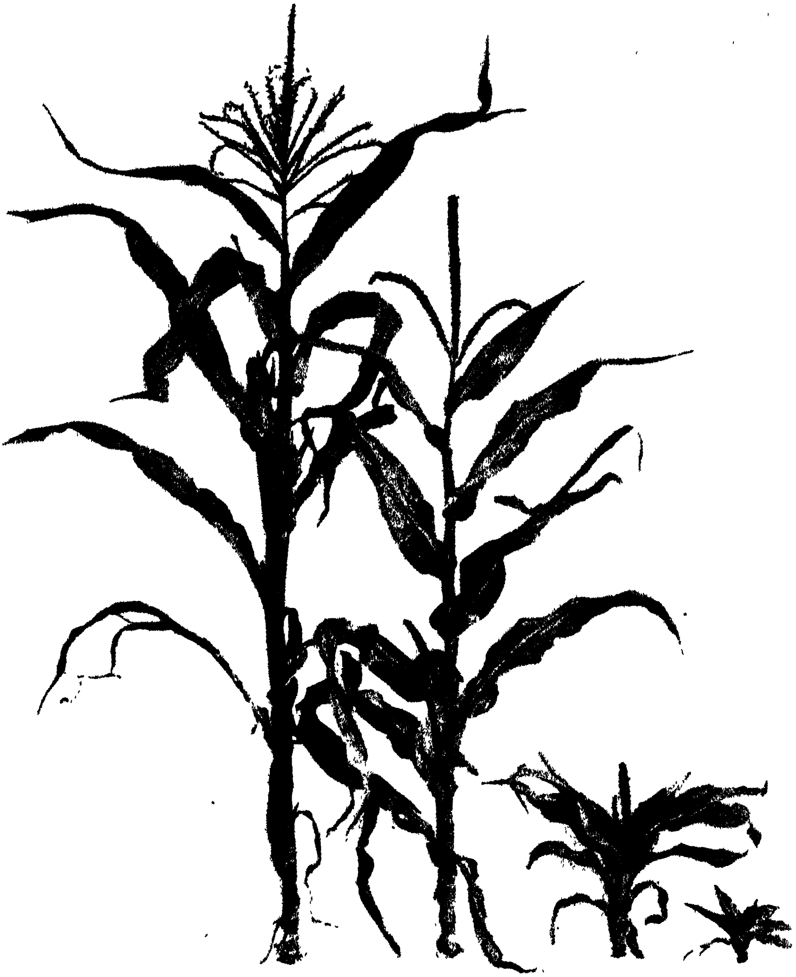

FIGURE 3.—Mature plants of normal, anther ear, dwarf, and dwarf anther ear.

broad Since the plant usually has several tillers which spread out almost on the ground, it sometimes has a total spread of branches and leaves of

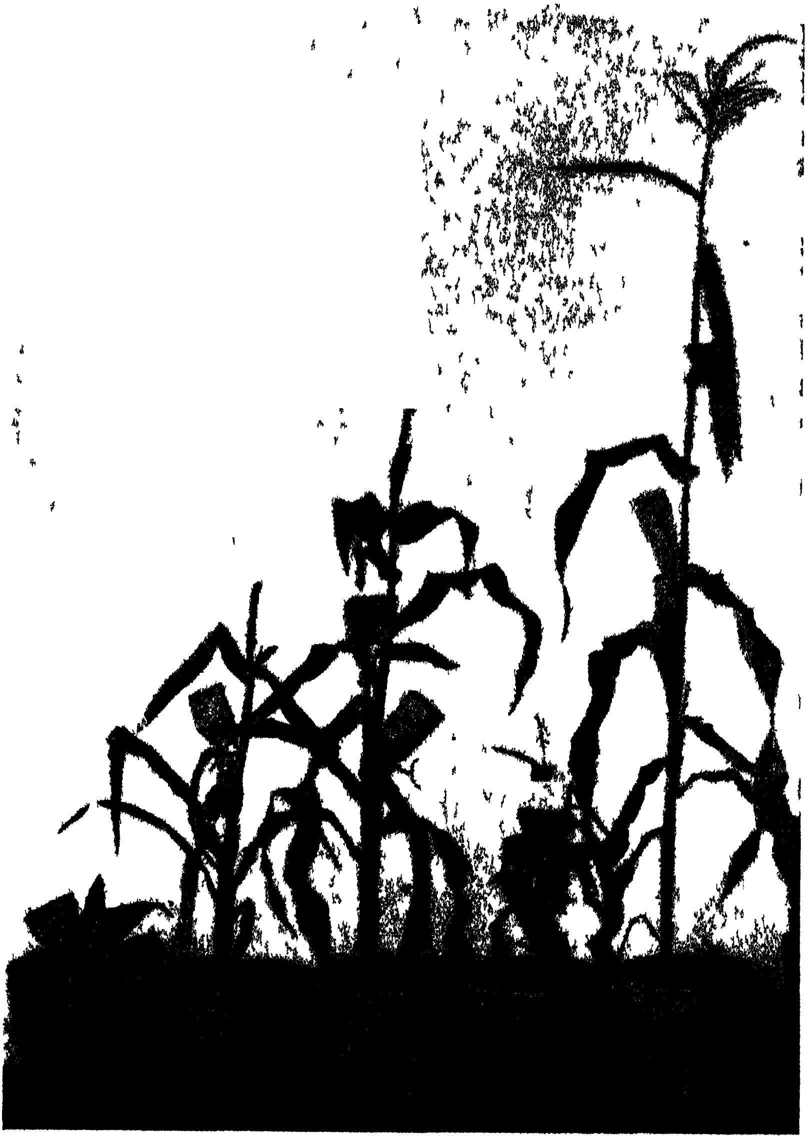

FIGURE 4—Mature plants of dwarf, small anther ear, larger anther ear, very small normal Tom Thumb, and tall normal.

two and a half times its height. The tassel is a short, compact, unbranched spike. The anthers do not shed pollen normally. No adequate test has

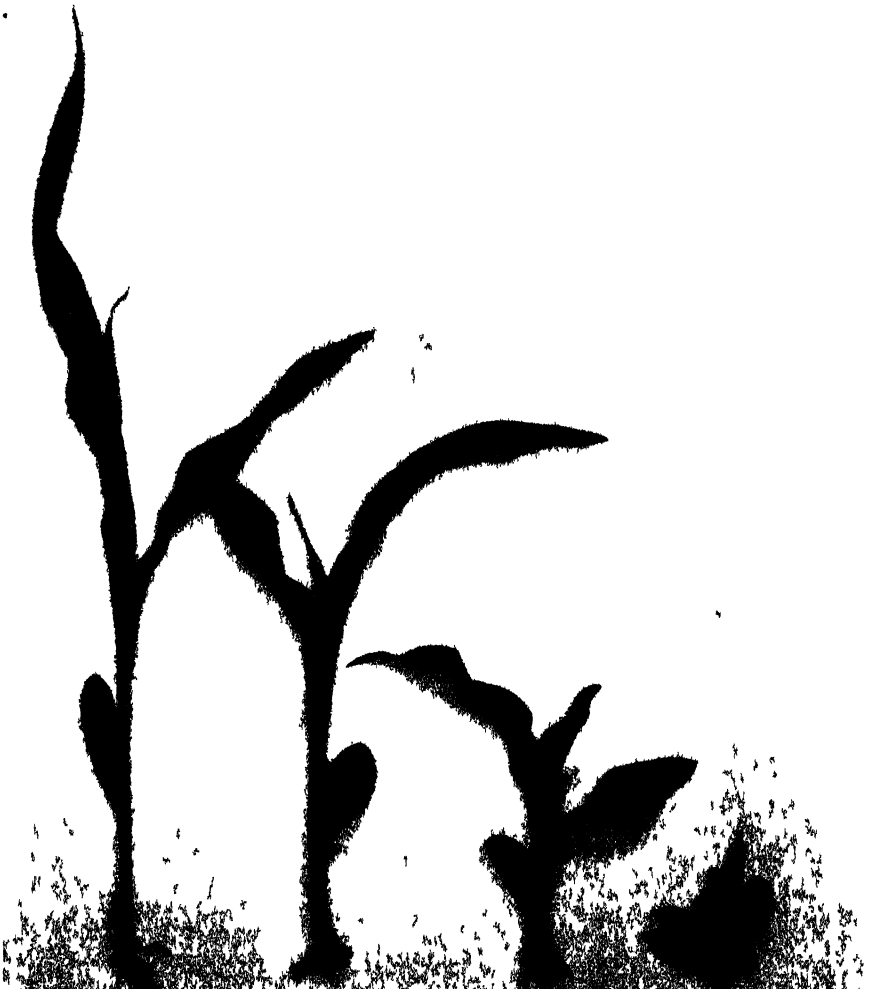

FIGURE 5.—Seedlings of normal, anther ear, dwarf, and dwarf anther ear.

been made to determine whether or not the pollen is functional. No fertile ear shoots have been observed on any of these plants.

INHERITANCE OF THE DWARF TYPE

That the dwarf type is inherited as a simple recessive to normal has been reported previously by the senior author (EMERSON 1911, 1912). Since in these accounts the anther-ear type was confused with the dwarf type, it seems wise to present here such evidence as is necessary to show the mode of inheritance of the true dwarf type in crosses between dwarf and normal.

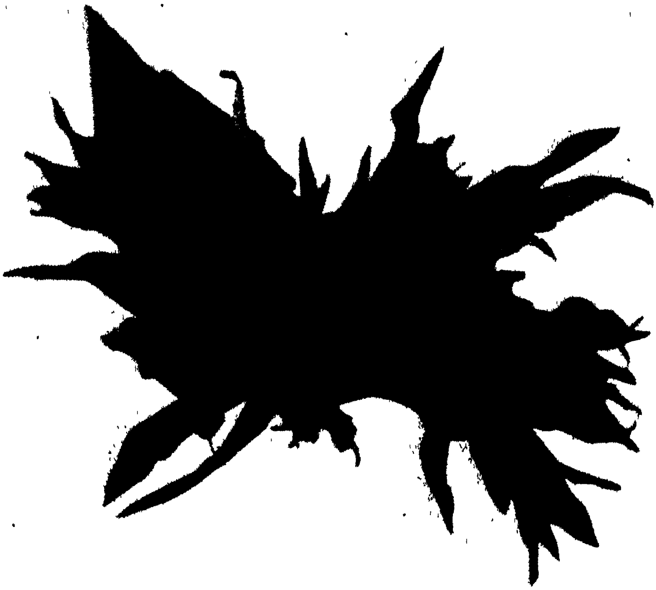

FIGURE 6.—Mature plant of dwarf anther ear.

Our records show that nineteen direct crosses of dwarf with normal resulted in 272 F_1 plants, all of which were normal. From selfed normal plants heterozygous for dwarf, fifty-four F_2 progenies with a total of 2932 individuals were grown. Of these F_2's thirty progenies were grown from seed planted in the field (table 7, group 1; see appendix). They resulted in 1095 normal and 231 dwarf plants. The deviation from a 3 : 1 ratio is 100.5 ± 10.6. The odds against such a deviation being due to chance are almost inconceivably great. The longitudinal axis of germinating seedlings of dwarf is relatively as short as that of mature plants.

Part of these F_2 cultures were grown at Lincoln, Nebraska, where it is customary to plant maize seeds about ten centimeters deep. The other cultures were grown at Ithaca, N. Y., from plantings not over three or four centimeters deep but in sticky clay soil. It seems obvious that under neither of these conditions could dwarf be expected to germinate well.

Fourteen other F_2 cultures (table 7, group 2) were germinated in light soil in greenhouse flats from seed planted about two centimeters deep. These cultures contained 436 normals and 138 dwarfs, a deviation from the 3 : 1 ratio of 5.5 ± 7.0. These two lots of F_2's were closely related, all having come from a single lot a few generations before, so that there can be little doubt that the same type is concerned in both. More decisive evidence, however, is afforded by the behavior of ten other F_2 cultures (table 7, group 3), each of which was planted both in the field and in the greenhouse. Of the field-grown lots, 277 were normal and 60 dwarf, a deviation from the 3 : 1 ratio of 24.3 ± 5.4, which should not occur by chance more than once in somewhat over 400 trials. Of the lots germinated in the greenhouse, there were 510 normals and 185 dwarfs, an excess of dwarfs over expectation of 11.3 ± 7.7, or such a deviation as might occur by chance about once in three trials.

F_1 plants of this cross, back-crossed with dwarfs, yielded eight progenies (table 8) with 331 normals and 354 dwarfs, a deviation from the expected equality of 11.5 ± 8.8, or such as might occur by chance almost twice in five trials. All but one of these eight progenies were germinated in the greenhouse.

Some of the F_2 normals remained constant in F_3 and others again threw dwarfs. Normals of the segregating F_3 progenies should theoretically be equivalent to F_2's. In all, seven F_3 and equivalent F_4 progenies (table 9, group 1) gave none but normal plants, a total of 208. Fifteen F_3 and F_4 progenies (table 9, group 2) gave 484 normals and 120 dwarfs, a deviation from the 3 : 1 relation of 31.0 ± 7.2, or such as might occur by chance only once in about 270 trials. All but three of the fifteen progenies were grown in the field. It is expected that one in three F_2 and equivalent F_3 normal plants will breed true. The seven true-breeding and fifteen segregating progenies is a close approach to this expectation.

Five F_2 and F_3 dwarfs bred true in the next generation (table 9, group 3) having produced a total of 56 dwarfs and one normal, the latter doubtless due to accidental pollination.

It is to be concluded, therefore, that dwarf, *d d*, behaves as a simple Mendelian recessive to normal, *D D*.

INHERITANCE OF THE ANTHER-EAR TYPE

That anther ear, like dwarf, is a simple Mendelian recessive to normal is shown by the data now to be presented. Eighteen F_1 progenies of crosses between anther ear and normal resulted in 485 normal plants. From such heterozygous normal plants, twenty-seven F_2 progenies with a total of 1151 individuals were grown. Ot these, twelve progenies grown in the field (table 10, group 1) had 455 normal and 108 anther-ear plants, a deviation from a 3 : 1 ratio of 32.8 ± 6.9, or such as would be expected to occur by chance not more than once in about 750 trials. Since anther ear is less vigorous than normal, it seems probable that this deficiency of anther ear was due to the failure of some plants to survive under field conditions. Thirteen F_2 progenies germinated in the greenhouse (table 10, group 2) contained 320 normal and 128 anther-ear plants, a deviation of 16.0 ± 6.2 from a 3 : 1 ratio. Such a deviation might be expected to occur by chance once in about twelve such trials. Two progenies (table 10, group 3) were grown in both the field and greenhouse. Of the field-grown plants there were 53 normal and 8 anther ear and of the greenhouse-grown ones 55 normal and 24 anther ear. The deviations from a 3 : 1 relation are 7.3 ± 2.3 and 4.3 ± 2.6, or such as might occur by chance about once in thirty and once in four trials, respectively.

Four normal plants of F_2 bred true in F_3 (table 11, group 1), three of them having produced a total of 272 normal plants from self-pollination and one of them 12 normals from a back-cross with anther ear. Seven F_2 normals and one F_3 equivalent to an F_2 (table 11, group 2) produced 393 normal and 110 anther-ear plants, a deviation from the 3 : 1 ratio of 15.8 ± 6.6, or such as might occur by chance about once in nine trials. The deficiency of anther ear is probably due to the fact that all but one of these cultures were field-grown.

Three F_2 anther-ear plants (table 11, group 3) produced 36 anther-ear and 2 normal plants in F_3. The normals are doubtless to be accounted for by accidental pollination, so that the conclusion that anther-ear plants breed true seems justifiable.

On the whole, therefore, anther ear, a_n a_n, can be said to behave in inheritance as a simple recessive to normal, A_n A_n.

GENETIC INTERRELATIONS OF THE DWARF AND ANTHER-EAR TYPES

In the foregoing account, it has been assumed that dwarf and anther ear are genetically different types. The evidence upon which this assumption is based is presented here. It has been stated previously that the two types were confused in the earlier papers of the senior author. Both types exhibit so much variation in stature and have so many characters in common,—stamens in the ears, relatively short culms, leaves, tassels and ears, and recessiveness to normal,—that this confusion was perhaps not unnatural in the early stages of the work.

Crosses of dwarf, d, with anther ear, a_n, have resulted in plants that were normal in all respects, including relatively long culms and leaves, slender much-branched tassels, and ears without stamens or with mere rudiments of stamens. Six such crosses (table 12) gave F_1 progenies totaling 83 normal plants. In addition normal plants heterozygous for dwarf, crossed with normals heterozygous for anther ear, resulted in 21 normal plants.

The F_2 progenies of the cross dwarf × anther ear, $D\ d\ A_n\ a_n$, are recorded in group 1 of table 13. A cross of a normal, $D\ D\ A_n\ A_n$, with an anther ear that is heterozygous for dwarf, $D\ d\ a_n\ a_n$, should give two F_1 genotypes, $D\ D\ A_n\ a_n$ and $D\ d\ A_n\ a_n$. Similarly a cross of a normal with a dwarf that is heterozygous for anther ear, $d\ d\ A_n\ a_n$, should give two genotypes, $D\ d\ A_n\ A_n$ and $D\ d\ A_n\ a_n$. The double heterozygotes, in case of each of these crosses, are equivalent to F_1's of the cross normal × dwarf anther ear and should give the same results in F_2 as F_1's of the cross dwarf × anther ear. Two such F_2 progenies are recorded in group 2 of table 13, the first being a cross of normal with anther ear and the second a cross of normal with dwarf.

The eight F_2 progenies had a total of 752 individuals distributed as shown below to the four types, normal, dwarf, anther ear, and dwarf anther ear. There is about one chance in thirteen that the deviations from the 9 : 3 : 3 : 1 relation, expected on the basis of two independent factor pairs, may be due to errors of random sampling, P equaling 0.076. The comparison follows:

	Normal	*Dwarf*	*Anther ear*	*Dwarf anther ear*	*Total*
Observed.	446	139	136	31	752
Calculated	*423*	*141*	*141*	*47*	*752*
Difference.	+23	−2	−5	−16	0

The deficiency of dwarf anther ear is doubtless due to the extremely small size of this type, which makes it incapable of germinating or of surviving under field conditions. Since both dwarf and anther ear are ordinarily deficient in field cultures, the much smaller double recessive could hardly be expected to survive in the field. The apparent excess of normals is probably due to the deficiencies of the other three types. That this explanation of the rather poor fit of the observed with the theoretical distributions is correct is made highly probable by the comparisons given below. Of the eight F_2 progenies, two were grown in the field only, three in the greenhouse only, and three in both the field and the greenhouse. Of the field-grown lots the deviations from the calculated frequencies are so great that they would not be expected to occur by chance more than about once in over twelve thousand trials, P equaling 0.000,085. With the greenhouse-grown lots, on the other hand, there is so close a fit between the observed and the theoretical frequency distributions that no value of P is to be found in published probability tables, χ^2 equaling 0.65. (When $\chi^2 = 1$ and $n' = 4$, $P = 0.80$.) The comparisons follow:

	Normal	*Dwarf*	*Anther ear*	*Dwarf anther ear*	*Total*
Field cultures					
Observed..	182	43	49	1	275
Calculated......	*155*	*52*	*52*	*17*	*276*
Difference......	+27	−9	−3	−16	−1
Greenhouse cultures					
Observed.. . ..	264	96	87	30	477
Calculated......	*268*	*89*	*89*	*30*	*476*
Difference. .	−4	+7	−2	0	+1

Three F_1 normals back-crossed with anther ear gave 157 normals and 93 anther ears (table 14, group 1). Numerical equality of these two classes is to be expected on the basis of independent inheritance of the complementary factor pairs *D d* and A_n a_n. The deviation from expectation is 32.0±5.3. This extreme deficiency of anther ear must be explained by the fact that all these cultures were field-grown, mostly under unfavorable conditions.

Five F_1 normals of the cross of dwarf with anther ear were back-crossed with dwarf and gave 274 normal and 204 dwarf plants (table 14, group 2). The deviation from equality is 35.0±7.4, which, while less than that found in the case of anther ear from back-crosses of F_1 with anther ear, is so great that appeal must be made here also to the difficulties to which dwarf is subjected under unfavorable field conditions.

The F_2 results of the cross dwarf × anther ear reported above, particularly the greenhouse-grown cultures, leave little doubt that *D d* and A_n a_n are independent complementary factor pairs. The crucial evidence, however, must be sought in the F_3 behavior of the several F_2 types. On the basis of the hypothesis under test, the F_2 types should behave as follows:

F_2 types		*F_3 behavior*			
Phenotype	Genotype	Normal	Dwarf	Anther ear	Dwarf anther ear
9 normal	1 $D\ D\ A_n\ A_n$	1			
	2 $D\ d\ A_n\ A_n$	3	1		
	2 $D\ D\ A_n\ a_n$	3		1	
	4 $D\ d\ A_n\ a_n$	9	3	3	1
3 dwarf	1 $d\ d\ A_n\ A_n$		1		
	2 $d\ d\ A_n\ a_n$		3		1
3 anther ear	1 $D\ D\ a_n\ a_n$			1	
	2 $D\ d\ a_n\ a_n$			3	1
1 dwarf anther ear	1 $d\ d\ a_n\ a_n$				1

Twenty-four F_2 normals were tested in F_3 (table 15). Plantings of most of the F_3 progenies (culture numbers S48-S90) were made in the greenhouse, and duplicate plantings were made in the field under unusually adverse soil conditions, part of them (S126-S166) in 1918 and part (9618-9642) in 1919. It was hardly to be expected that the weaker types would appear in field plantings in numbers close to the theoretical ones. The field and greenhouse plantings are recorded together in table 15.

Of the twenty-four F_2 normals tested, two (table 15, group 1) remained constant, $D\ D\ A_n\ A_n$, in F_3, having produced 290 normal plants and no other types.

Five F_2 normals, $D\ d\ A_n\ A_n$, (group 2) produced 374 normals and 86 dwarfs, a deviation from the 3 : 1 relation of 29.0 ± 6.3. Such a deviation could occur by chance not more than once in over five hundred trials. Of these 460 F_3's, the 173 grown in the greenhouse consisted of 120 normals and 53 dwarfs, an excess over expectation of 9.8 ± 3.8 dwarfs, a deviation that might occur by chance about once in thirteen trials.

Three F_2 normals, $D\ D\ A_n\ a_n$, (group 3) produced 249 normals and 61 anther ears in F_3, a deviation from the 3 : 1 relation of 16.5 ± 5.1, or such as might occur by chance not more than three times in one hundred trials. Of these 310 F_3's, the 132 greenhouse-grown plants consisted of 98 normal and 34 anther-ear plants, a deviation of only 1.0 ± 3.4 from the 3 : 1 ratio.

Fourteen F_2 normals, $D\ d\ A_n\ a_n$, (table 15, group 4) produced in F_3 the three types, normal, dwarf and anther ear, and six of them produced dwarf anther ear in addition. In a total of 1174 F_3 plants, the four types, in the order given above, appeared in the numbers 774 : 174 : 215 : 11.

The departures from expectation are so extreme that no value of P is to be found in published tables, χ^2 equaling 84. (When $\chi^2 = 30$ and $n' = 4$, $P = 0.000,001$). Of these 1174 F_3 plants, 594 that were germinated in the greenhouse exhibited the four types in the numbers 330 : 105 : 148 : 11. Even in case of these greenhouse-grown seedlings, there is not more than one chance in a million that the observed deviations from expectation could be due to errors of random sampling. The great deficiency in the very weak double recessive, dwarf anther ear, is responsible more than anything else for the poor fit. All the plants of this type that appeared were in greenhouse cultures but apparently they were too weak to complete their germination even under greenhouse conditions. The only other marked difference between observed and calculated frequencies in these greenhouse cultures occurred in the anther-ear class, but here there was an excess over expectation. It is not particularly difficult to separate the smaller types of anther ear from normal even in the seedling stage, but the taller anther-ear stocks cannot always be distinguished until the flowering stage. It seems possible that the recorded excess of anther ear in these lots may have come in part from errors in classification.

Notwithstanding the poor fit of observed with calculated frequencies in some cases, the F_2 normals of the cross of dwarf with anther ear exhibited in general the F_3 behavior expected of them in accordance with the hypothesis that $D\ d$ and $A_n\ a_n$ are complementary independently inherited factor pairs. Of the twenty-four F_2 normals tested, the four types of F_3 behavior, were exhibited in the relation of 2 : 5 : 3 : 14, while the theoretical relation for twenty-four individuals is 2.7 : 5.3 : 5.3 : 10.7. There is slightly better than an even chance that deviations such as this might occur through errors of random sampling.

Two F_2 dwarfs were tested by their F_3 behavior, both in greenhouse plantings. One of these, $d\ d\ A_n A_n$, (table 15, group 5) bred true, having produced 51 dwarf plants, and one, $d\ d\ A_n\ a_n$, threw 17 dwarfs and 6 dwarf anther ears, as nearly a 3 : 1 ratio as is possible with a total of 23 plants. Theoretically, one out of three F_2 dwarfs should breed true and two throw dwarfs and dwarf anther ears.

No F_2 anther ear was tested in F_3. It is obviously impossible to test dwarf anther ear, since no plant of that type has ever produced seeds.

It has been shown (table 14) that F_1 normals of the cross dwarf × anther ear back-crossed with anther ear throw only normal and anther ear in the next generation. Of such normals, half, $D\ D\ A_n\ a_n$, should again throw normal and anther ear, and half, $D\ d\ A_n\ a_n$, should throw all four types. Records of twenty-one such tests are given in table 16. Eleven normals threw normal and anther ear (table 16, group 1) in the relation of 252 : 63, a deviation from 3 : 1 of 15.8 ± 5.2. Only one of these progenies was germinated in the greenhouse and this consisted of 73 normal and 24 anther ear, deviation 0.3 ± 2.9. The other ten normals tested (group 2) gave a total of 257 plants distributed to the four types in the relation of 186 : 26 : 43 : 2. This extreme departure from the expected 9 : 3 : 3 : 1 relation is doubtless due to the fact that all but one of the cultures were field-grown. This one showed the four types in the relation of 10 : 3 : 3 : 2, a very close fit to expectation, χ^2 equaling 0.83. Of the 21 normals tested, 11 gave the one type of behavior and 10 the other, where equality was expected.

Nine anther-ear plants of these same back-crosses (table 8) were tested by their F_3 behavior. Four bred true, $D\ D\ a_n\ a_n$, with a total of 57 anther-ear plants (table 16, group 3) and five, $D\ d\ a_n\ a_n$, produced anther ear and dwarf anther ear only (group 4) in the relation of 127 : 37, a deviation from 3 : 1 of 4.0 ± 3.7. All these lots were grown in the greenhouse. Since half of the anther-ear plants tested are expected theoretically to breed true and half to throw the two types observed, the results are in the closest possible accord with expectation.

F_1 normal plants of the cross dwarf × anther ear back-crossed with dwarf (table 14), it will be recalled, threw normals and dwarfs only. Thirteen of these normals were tested by their progenies. Half of such normals, $D\ d\ A_n\ A_n$, should throw normal and dwarf, and half, $D\ d\ A_n\ a_n$, should throw the four types. Seven of the thirteen (table 17, group 1) threw a total of 161 normal and 26 dwarf, a deviation from 3 : 1 of 20.8 ± 4.0. The deficiency of dwarfs was doubtless due to the fact that all the cultures were field-grown. The other six normals that were tested (table 17, group 2) threw normal, dwarf, and anther ear in the relation of 115 : 10 : 19. The failure of dwarf anther ear to appear, as well as the extreme departure from the expected 9 : 3 : 3 : 1 relation, is ascribed to the field conditions under which all of these cultures were grown.

Three dwarfs from the same back-crosses (table 14) were tested by greenhouse progenies and found (table 17, group 3) to breed true, having produced a total of 173 dwarf plants. Half of them, $d\ d\ A_n\ A_n$, were expected to breed true and half, $d\ d\ A_n\ a_n$, to throw dwarf and dwarf anther-ear. While neither of these dwarfs threw dwarf anther-ear, it has been shown that some such dwarfs carry the a_n factor in a heterozygous condition, $d\ d\ A_n\ a_n$. Dwarfs of back-cross progenies when crossed with normals gave normals in F_1. One of these F_1 normals (table 13, group 2) threw the four types in F_2 with frequencies of 47 : 12 : 13 : 4. This behavior could have resulted only from a dwarf of the genotype *d d* A_n a_n.

By way of summary, it may be noted that all the genotypes theoretically possible on the assumption of two independently inherited factor pairs, *D d* and A_n a_n, have been demonstrated in the cross of dwarf with anther ear. It is true that the observed frequency distributions of the several phenotypes have in many instances been far from the theoretical frequencies, but it has been shown conclusively, by growing cultures in both the field and the greenhouse, that these departures from expectation have been due to the failure of the weaker forms to germinate or to survive under field conditions. In a few cases, an excess of anther ear over expectation has been recorded with cultures germinated in the greenhouse, but this result is regarded as due mainly to the difficulty of separating anther ear from normal in the seedling stage.

VARIABILITY IN STATURE OF DWARF AND ANTHER-EAR TYPES

In the foregoing sections of this paper, repeated references have been made to the differences in height between different stocks of anther ear. Similar differences exist between diverse stocks of dwarf. Since all our stocks of dwarf and of anther ear came each from a single plant, it is of interest to consider the possible nature of these diversities.

The dwarf type ordinarily varies in height from about 30 to 60 centimeters as contrasted with heights of from about 120 to 200 centimeters for normal plants of the same cultures. Frequency distributions for height of plants of F_2 progenies of crosses of dwarfs with normals are given in table 1. In no case have the heights of dwarfs and of normals of the same culture been observed to overlap. This sharp segregation in height is ascribed to the single factor pair *D d*. That other growth factors, and environmental influences as well, affect the height of dwarfs, much as they do the height of normal plants, is evident from crosses of dwarfs, *d d*, with normals *D D*, of very low stature, such as Tom Thumb pop.

TABLE 1

Heights of normal and dwarf plants of F_2 progenies of the cross dwarf × normal.

PEDIGREE CULTURE	HEIGHT OF PLANTS IN DECIMETERS																		
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean
396 Normal...										1	1	3	5	2	2	5	2		16 0
Dwarf .	1	1																	3 5
7424 Normal ..										2	3	7	5	6	7	4		2	15.6
Dwarf.. .	11	23	12	3															4 1

It has been shown (EMERSON and EAST 1913) that, when a tall normal strain is crossed with Tom Thumb, the height of the F_1 plants is intermediate between the height of the parents, while the range in height of the F_2 plants extends practically to the extremes of both parents. An illustration of this behavior is given in table 2. Such behavior in F_2 is ordinarily interpreted on the basis of partially dominant multiple factors affecting growth. Obviously no single factor pair is alone concerned here, of the nature of *D d*, which is assumed to be responsible for the sharp segregation shown in table 1.

TABLE 2

Heights of parental, F_1, and F_2 plants of the cross tall dent × Tom Thumb pop.

PEDIGREE CULTURE	HEIGHT OF PLANTS IN DECIMETERS																		
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	Mean
357 Tall dent											2	1	2	6	2	4	3	3	20.9
508 Tom Thumb	2	6	5	2															8.5
509 F_1 . . .									1	3	5	7	4						17.5
510 F_2 .	2			2	8	6	25	26	29	30	32	28	22	6	4	1	2		15.9

When dwarf plants, *d d*, are crossed with normals, *D D*, of very low stature like Tom Thumb pop, both the normals and dwarfs differentiated by *D d* should be influenced by diverse partially dominant growth factors just as is true of the F_2 plants of crosses of tall normals with the small Tom Thumb. The results of such a cross are presented in table 3. For ready comparison, the dwarfs of culture 7424 (table 1) and Tom Thumb plants of culture 508 (table 2) are included. Neither of these cultures was grown during the same season as the F_1 and F_2 progenies of the cross of dwarf with Tom Thumb. It will be noted that the F_1 plants of this cross were taller than either parent. Evidently the dwarf parent of the cross brought into the combination partially dominant growth factors that

TABLE 3

Heights of Tom Thumb and dwarf plants and of F_1 and F_2 of the cross dwarf × Tom Thumb.

PEDIGREE CULTURE	HEIGHT OF PLANTS IN DECIMETERS																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Mean
508 Tom Thumb							2	6	5	2								8.5
7424 Dwarf . .			11	23	12	13												4.1
10727 F_1 normal .											1	2		4	2	1	1	14.0
10728 F_2 normal. . .						1	4	3	4	4	6	14	13	12	2	4		11.6
F_2 dwarf.	1	8	6															2.3

were not present in Tom Thumb. These together with the dominant factor *D* from Tom Thumb resulted in a height growth not greatly different from that of F_1 plants of crosses between Tom Thumb and tall normals. If this supposition is correct, the F_2 plants should exhibit a wide range of variation in height, some of the F_2 normals being taller than the normal Tom Thumb parent and some of the dwarfs being shorter than the dwarf parent. This expectation was strikingly realized. The F_2 normals ranged in height from that of Tom Thumb as ordinarily grown to nearly twice that height with a mean height considerably above that of Tom Thumb. The F_2 dwarfs did not show a great range in height, due perhaps to the small numbers involved, but their mean height was considerably below that of the usual height of dwarfs. While the ranges of heights of the F_2 normals and dwarfs did not overlap, they came close together. Moreover, the shortest F_2 normals were no taller than the tallest dwarfs of other cultures. Evidently the height of these F_2's was influenced by growth factors besides *D d*.

Anther-ear plants also are ordinarily shorter than normal plants of the same cultures. In some cultures anther ear is sharply differentiated from normal by its semi-dwarfness (table 4, culture 294), there being no overlapping of the height distributions of the two forms. In such cultures the principal factor pair for plant height differentiating normal from anther ear is $A_n\ a_n$. Since $a_n\ a_n$ does not exert so strong a dwarfing effect as does *d d*, there is less difference in height between normals and anther ears even of such a culture as 294 than there usually is between normals and dwarfs. In other cultures the anther-ear plants, while on the average shorter than normals, overlap them in height distributions so much that separation of the two forms must be based on characters other than height (table 4, cultures 298 and 7421).

TABLE 4

Heights of normal and anther-ear plants of F_2 progenies of the cross anther ear × normal.

PEDIGREE CULTURE	HEIGHT OF PLANTS IN DECIMETERS																	
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean
294 Normal......								2	5	5	11	4	3	1	1			18.9
Anther ear..	1	5	1	1	1													10.6
298 Normal.......				1		1	3	4	6	8	8	8	8	3	1			18.6
Anther ear...	1	1			2	5	2	1	2	1								14.0
7421 Normal.... .						1			4	1	3	13	9	9	5	4	2	21.1
Anther ear....					1	1		3	4	4	1	5	1	1				17.9

The cause or causes of overlapping in the height ranges of normal and anther-ear plants of some cultures and the sharp segregation in height of these two types in other cultures cannot be determined with certainty from the data now available. Certain possibilities, however, should be noted.

There may exist a series of allelomorphs instead of the single pair $A_n\ a_n$. The fact that all our anther-ear stocks came originally from a single plant makes this supposition improbable. Since, however, this plant was not self-pollinated, the suggestion is not an impossible one. Moreover, there is always the possibility of one or more mutations having occurred in one or both members of the $A_n\ a_n$ pair since the original stock was obtained. If there exist an allelomorph of $A_n\ a_n$ which has a less dwarfing effect than a_n, just as a_n has less effect than d, the anther-ear plants of some cultures might well be considerably taller than those of other cultures without any corresponding difference in height between the normal plants of the two lots.

A second possibility is that other growth factors may be linked with the $A_n\ a_n$ pair. If multiple factors affecting height growth are distributed widely throughout the chromosome complex of maize, as seems probable, some of them must necessarily be linked with $A_n\ a_n$. Such linkage might well bring about overlapping of heights of normal with those of anther-ear plants in certain cultures and sharp segregation of heights in others, the result in any one culture depending on whether certain dominant or partially dominant growth factors entered the cross with A_n or with a_n. The effects of such linkage might of course be more or less masked by the presence in a heterozygous condition of other growth factors not linked with $A_n\ a_n$.

While it seems highly probable that some growth factors may be linked with $A_n\ a_n$, resort to this hypothesis is not essential to a plausible explanation of the data presented in table 4. It may well be that in such sharply segregating cultures as 294 few growth factors other than $A_n\ a_n$ were heterozygous while in such overlapping cultures as 298 and 7421 numerous other growth factors were heterozygous. In case of the latter cultures, such normal plants as happened to receive few dominant or partially dominant growth factors would be expected to be relatively short in stature and such anther-ear plants as happened to receive many of these factors would be relatively tall, thus insuring an overlapping of the height distributions of the two types. If this hypothesis be true, both normal and anther-ear plants should exhibit greater variability in height in overlapping cultures than in sharply segregating ones. While the numbers of individuals involved were perhaps too few to afford conclusive evidence on this point, it is apparent that the overlapping cultures of table 4 were more variable than the sharply segregating culture.

As bearing further upon this suggestion of the possible effect of heterozygous growth factors in addition to $A_n\ a_n$, a cross of the very small normal race Tom Thumb with a stock of anther ear of relatively low stature may be cited. Unfortunately individual records of height of plants were not made for this cross, our records indicating merely the range of variation in height of the F_2 normals and anther ears. The F_1's of this cross, culture 1848, were normals of medium height such as result from crosses of Tom Thumb with a tall normal race. The ranges in height of the F_2 normals and anther ears are shown in table 5. For comparison the heights of anther-ear plants of culture 294 (table 4), of Tom Thumb plants of culture 508 (table 2), and of dwarf plants of culture 7424 (table 1) are included. The added cultures were not grown at the same time as the F_2 cultures and are not therefore wholly comparable with them.

TABLE 5

Range of heights of parent types and of F_2 normal and anther-ear plants of the cross anther ear × Tom Thumb, and of a culture of dwarf plants.

PEDIGREE CULTURE	HEIGHT OF PLANTS IN DECIMETERS													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
294 Anther ear (table 4)							←	—	—	—	→			
508 Tom Thumb (table 2)					←	—	—	→						
7424 Dwarf (table 1)	←	—	—	→										
8286 F_2 normal				←	—	—	—	—	—	—	—	—	—	→
F_2 anther ear			←	—	—	—	—	—	—	—	—	—	→	

It is evident from the height distributions shown in table 5 that an unusually large complex of growth factors besides $A_n\ a_n$ were brought into this cross by Tom Thumb pop and the anther-ear parent. The anther-ear plants of F_2 ranged in height from above the usual height of the stock of anther ear used as one parent to much below the ordinary height of Tom Thumb and even to about the average height of ordinary dwarfs. The F_2 normals showed about the same range of heights but were on the whole slightly taller than the anther ears.

From the above it seems safe to conclude that the diverse heights of different anther-ear stocks, and of dwarfs as well, are in large measure due to the influence of growth factors other than $A_n\ a_n$ and $D\ d$. The overlapping in height of anther-ear and normal plants of some F_2 cultures

TABLE 6

Heights of F_2 normal, anther ear, dwarf and dwarf anther ear of the cross dwarf × anther ear and of a cross equivalent to normal × dwarf anther ear

PEDIGREE CULTURE	HEIGHT OF PLANTS IN DECIMETERS																									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Mean
7420 Normal												4	5	3	10	9	4	5	6							15.7
Anther ear										2	2	1	1	1		1										11.3
Dwarf			[illegible]	6		1																				4.1
Dwarf anther ear	1																									
10536 Normal												1		2	3	7	14	7	9	2	8	2	5	2	1	18.6
Anther ear									2		2		2	2	2	6	1		2	1						14.8
Dwarf		4	[illegible]	2	1																					
10735, 10736 Normal														1	2	3	5	3	5	[illegible]	3					18.4
Anther ear											2		1	1			1	[illegible]	2							15.6
Dwarf				[illegible]																						3.5
Dwarf anther ear	1																									

and the sharp height segregation of these types in other cultures appears to be due largely to the relative number of growth factors that happen to be in a heterozygous condition in the several cases. The linkage of certain growth factors with A_n a_n probably has an effect also. Whether allelomorphs of A_n a_n are involved cannot be determined from the data at hand, but that the pairs A_n a_n and D d, particularly the latter, play an important part in determining height of plant is evident. In no single F_2 culture as yet observed have dwarfs overlapped in height either normals or anther ears.

That dwarfs are distinctly smaller than the other two types, normal and anther ear, is particularly well shown in table 6 in which are presented data from F_2 cultures involving all these types together with a few plants of the double recessive dwarf anther ear. One of these cultures was an F_2 of a cross of normal with anther ear heterozygous for dwarf, equivalent to a cross of normal with dwarf anther ear, and the others were F_2's of the cross dwarf × anther ear (table 13). The normals in all cases were somewhat taller than the anther ears. This increase in height over the parent types, dwarf and anther ear, is doubtless due in the main to the interaction of the dominant factors D and A_n. The sharp segregation in height of dwarfs is in strong contrast with the overlapping of the heights of normal and anther ear. So few dwarf anther ears were measured that it cannot be determined from the data whether dwarf and dwarf anther ear ever overlap in height ranges as do normal and anther ear.

RUDIMENTARY STAMENS IN DWARF AND ANTHER-EAR TYPES

The original stocks of dwarf and of anther ear had well developed stamens throughout the ears. In 1915 in a culture consisting of normals and dwarfs, the ears of some of the dwarf plants were found to have only rudimentary stamens. Since other dwarfs of the same culture had well developed stamens, the character was evidently heterozygous in the stock. A dwarf with rudimentary ear stamens was crossed with an anther ear with normal ear stamens. The F_1 plants had no stamens in their ears or only a few very rudimentary ones such as are sometimes seen in normal stocks. The F_2 cultures of this cross (table 13) consisted of normal, dwarf, anther ear and dwarf anther ear. The latter had no ears and the normals were without ear stamens. Of the F_2 dwarfs and anther ears some had well formed ear stamens and others only rudimentary ones. The 64 dwarf and anther-ear plants were distributed among the four classes as follows:

	Stamens normal	*Stamens rudimentary*	*Total*
Dwarf	11	22	33
Anther ear	21	10	31
Total	32	32	64

While the number of individuals observed is too small for reliable indications, the frequency distribution noted above suggests that a factor for rudimentary stamens in this material may be linked with *d*. But no simple linkage relation of that kind would be expected to give the observed frequency distribution in an F_2 culture. If a factor for rudimentary stamens should be found to behave as a dominant in dwarf and as a recessive in anther ear, the observed F_2 frequencies should be fairly closely realized. Until the question is investigated more fully, no purpose is served by further speculation.

SUMMARY

Two andromonoecious types of maize, dwarf and anther ear, are described and compared with normal maize and with the double recessive, dwarf anther ear. Both types are characterized by the presence of stamens,—usually well developed but in some stocks rudimentary,—throughout the ear. These types have relatively short internodes and relatively short, broad leaves. The tassels are stout and relatively little branched. The ears of both types are short and comparatively thick and frequently end in unbranched tassel-like spikes. With respect to all these characters except perfect flowers the dwarf type is more extreme than the anther-ear type, the latter, in some stocks, approaching closely to the normal type of maize. Dwarf anther ear is an extremely dwarf type with very broad, short leaves and thick, unbranched tassels. No pollen is shed and no ear shoots have been produced by this type.

Both dwarf and anther ear are simple recessives to normal. Intercrosses of these two types give normal plants in F_1 and normals, dwarfs, anther ears, and dwarf anther ears in F_2 in approximately a 9 : 3 : 3 : 1 relation. Owing to failure of the more dwarf forms to germinate or to persist under field conditions, this numerical relation of the several types is approached closely only in greenhouse cultures. This F_2 behavior is interpreted on the basis of two independent factor pairs, $D\ d$ and $A_n\ a_n$. The hypothesis has been tested by the behavior in F_3 and later generations of the several types and by intercrosses between them.

Marked diversities have been observed in height of plants between different anther-ear stocks and to a less degree between different dwarf stocks. These diversities are ascribed to the influence of partially domi-

nant growth factors other than D d and A_n a_n, such as are assumed to differentiate tall and short races of normal maize.

Dwarf plants with rudimentary ear stamens crossed with anther-ear plants with fully developed stamens have given in F_2 both dwarf and anther ear with both rudimentary and well developed ear stamens. The numerical relation of these four types is not that expected on the basis either of independent inheritance or of any simple linkage relation.

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APPENDIX—TABLES

TABLE 7

F_2 progenies of the cross dwarf × normal.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_2 PLANTS			
	F_1	F_2	Field		Greenhouse	
			Normal	Dwarf	Normal	Dwarf
1	164 – 4	396	22	2		
	1794– 3	2237	63	19		
	1799– 6	2226	15	3		
	9	2221, 2222	22	11		
	14	2225	16	3		
	28	2224	13	3		
	32	2220	15	5		
	37	2223	12	4		
	1914– 1	1946	37	6		
	2	1952	34	2		
	3	1948	37	3		
	4	1953	49	4		
	5	1947, 1949	70	7		
	8	1945	38	7		
	9	1944	41	3		
	10	1950	25	4		
	11	1954	47	10		
	12	1951	36	1		
	2403– 1	2962–2966	34	7		
	2967– 2	6826, 6827	71	20		
	10	6830, 6831	38	9		
	11	6828, 6829	112	29		
	4257– 1	5539, 5540	41	16		
	2	5541, 5552	61	14		
	11	5536–5538	7	3		
	9613– 8	10728, 10729	67	15		
	9643– 6	10400	19	3		
	8	10401, 10731	23	7		
	A1400–4	10730	19	5		
	87–4	7697	11	6		
	Total, 30 progenies		1095	231		

Table 7 (*continued*)

F_2 progenies of the cross dwarf × normal.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_2 PLANTS			
	F_1	F_2	Field		Greenhouse	
			Normal	Dwarf	Normal	Dwarf
2	1120— 1	1451			32	15
	2	1452			35	9
	3	1453			39	10
	4	1454			37	12
	3608—18	6780			18	4
	7351— 5	S220—S222			84	23
	7859— 1	S294, S295			39	13
	7867— 1	S289—S291			12	8
	2	S292, S293			15	5
	7869— 2	S298, S299			16	6
	7870— 1	S300			38	9
	7871— 1	S301, S302			30	9
	S6—2	7759, S200, S201			26	8
	6	7760, S202, S203			15	7
	Total, 14 progenies				436	138
3	3609—18	6779, 7408	15	1	30	13
	4257— 3	5535, 6784	62	10	73	27
	21	5543, 6740, 6783	48	19	75	18
	7417— 6	S217	13	5	67	29
	7	S218	6	0	27	8
	7440— 1	S190	30	3	26	12
	2	S191	25	11	37	12
	3	S192	35	1	37	13
	5	S195	32	7	35	13
	S7—7	7434, S189	11	3	103	40
	Total, 10 progenies		277	60	510	185
	Total, 54 progenies		1372	291	946	323

Table 8

F_2 progenies of the cross dwarf × normal, back-crossed with dwarf

PEDIGREE NUMBERS		NUMBER OF F_2 PLANTS	
F_1 × dwarf	F_2	Normal	Dwarf
6785—1 × 6784—18	7429, 7430, S179	37	44
1 × 28	7431, 7432	63	60
S180—1 × S300—3	8857	14	16
S184—4 × S202—1	8858, 8859	89	98
8 × 1	8860—8863	51	46
S186—2 × S294—2	8855, 8856	17	22
S207—6 × A744—11	9643	8	4
S219—2 × S222—7	8865—8868	52	64
Total, 8 progenies		331	354

TABLE 9

F_3 and equivalent F_4 and F_5 progenies of the cross dwarf × normal.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_3, F_4 AND F_5 PLANTS	
			Normal	Dwarf
1	F_2	F_3		
	1945–18	2423	13	
	2220–14	4208	30	
	6829– 9	7655–7657	25	
	F_3	F_4		
	5049–13	6913, 6914	11	
	37	6919–6921	78	
	5054–10	6745, 6872	41	
	5055– 2	6871, 7315	10	
	Total, 7 progenies		208	
2	F_2	F_3		
	1945–10	2308	8	3
	11	2305, 2306	16	7
	1946–34	2523, 2524	39	11
	1949–43	2535, 2536	12	13
	2966– 7	5049–5055	86	15
	6828–12	7658–7660	24	1
	F_3	F_4		
	5049–25	6816–6818	65	19
	5050– 1	6874	13	4
	6	6822–6824, 7058, 7059	79	12
	5052– 3	6833	18	6
	7	6825, 7202, 7323, 7442	43	4
	12	6832	21	11
	5053– 1	6875, 6911	37	9
	7660– 1	8536	8	3
	F_4	F_5		
	6832–31	7313, 8181	15	2
	Total, 15 progenies		484	120
3	F_2	F		
	7429–18	8213, 8214		4
	7760– 9	8206		5
	9643– 3	10402, 10732		12
	8189–50 × 8217–27	9644		20
	F_3	F_4		
	5052– 5	85	1	15
	Total, 5 progenies		1	56

TABLE 10

F_2 progenies of the cross anther ear × normal.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_2 PLANTS			
			Field		Greenhouse	
	F_1	F_2	Normal	Anther ear	Normal	Anther ear
1	19C −2	294−296	38	8		
	19E −2	297−299	59	15		
	419 −3	1019−1021	26	8		
	432 −1	1037	31	6		
	4	1040	146	30		
	2451−1	4089	8	2		
	3	4088	8	3		
	4078−9	6781, 6782	30	15		
	6786−1	7434, 7435 8279, 8280	63	12		
	5	7436, 7437	12	4		
	7874−1	8282, 8283	19	3		
	7875−1	8286	15	2		
	Total, 12 progenies		455	108		
2	792 −4	1439			12	5
	796 −3	1442			23	19
	799 −3	1443			29	9
	800 −2	1445			6	1
	801 −1	1446			32	13
	803 −1	1448			53	16
	2	1447			32	12
	824 −1	1438			36	14
	3	1462			23	7
	8272−2	9763			24	8
	8273−3	9764			29	17
	8275−3	9765			11	3
	8278−1	9766			10	4
	Total, 13 progenies				320	128
3	792 −2	1440, 1847	30	5	25	8
	800 −1	1444, 1764, 1843	23	3	30	16
	Total, 2 progenies		53	8	55	24
Total, 27 progenies			508	116	375	152

TABLE 11

F_3 and equivalent F_4 progenies of the cross anther ear × normal.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_3 PLANTS	
			Normal	Anther ear
1	F_2	F_3		
	295 –1	810–812, 1766	53	
	297 –3	816–820	105	
	1843–3	2241	114	
	7410–1 × 7436–7	S269	12	
	Total, 4 progenies		284	
2	294 – 1	805, 806	33	3
	296 – 2	814	17	5
	297 – 1	821–823, 1839	12	3
	1843– 5	2243	110	35
	11	2242	61	17
	12	2240	66	16
	1847–16	2244	69	21
	F_3	F_4		
	805 –3	1449	25	10
	Total, 8 progenies		393	110
3	294 –28	746, 784, 1123		7
	6781–22	7409		15
	6782–16	7410	2	14
	Total, 3 progenies		2	36

TABLE 13

F_2 progenies of the cross dwarf × anther ear and crosses equivalent to normal × dwarf anther ear.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_2 PLANTS							
			Field				Greenhouse			
	F_1	F_2	Normal	Dwarf	Anther ear	Dwarf anther ear	Normal	Dwarf	Anther ear	Dwarf anther ear
1	7407–1	S176					25	9	8	0
	5	10735, S177	20	5	8	1	18	11	9	6
	9	10736, S178	25	2	7	0	31	10	8	1
	7418–3	S174	31	4	6	0				
	6787–1	7420, S97	23	7	6	0	39	14	15	5
	S3–3	9754					104	40	34	14
	Total, 6 progenies		99	18	27	1	217	84	74	26
2	9161–5	10536	83	25	22	0				
	S224–2	9761					47	12	13	4
Total, 8 progenies			182	43	49	1	264	96	87	30

TABLE 12

F_1 progenies of the cross dwarf × anther ear.

PEDIGREE NUMBERS		NUMBER OF F_1 PLANTS NORMAL
P_1	F_1	
2451− 2 × 2440− 2	4084	2
5053− 3 × L185− 1	6787, 6788, S3, S4	29
6779−23 × 6781−22	7407	10
6781− 3 × 6831−14	7418, S175	35
6783−16 × 6781−18	7419	4
7409−14 × 7439−12	S276	3
Total, 6 progenies		83

TABLE 14

F_2 progenies of the cross dwarf × anther ear back crossed with anther ear and with dwarf

GROUP	PEDIGREE NUMBERS		NUMBER OF F_2 PLANTS		
	F_1 × Anther ear	F_2	Normal	Anther ear	Dwarf
	7418− 5 × 7409− 7	S167, S168	54	31	
	13 × 7	S169	36	17	
1	S3−5 × 6781−27	7421−7423, S98	67	45	
	Total, 3 progenies		157	93	
	F_1 × dwarf				
	7418−11 × 7439−21	S91, S170, S171	45		16
	13 × 21	S92, S172, S173	128		85
2	S4−1 × 6827−10	7427, 7428, S100	25		31
	S5−15 × S4−5	7424−7426, S99	26		30
	S175−15 × S200−2	8864	50		42
	Total, 5 progenies		274		204

TABLE 15

F_3 progenies of the cross dwarf × anther ear.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_3 PLANTS			
	F_2	F_3	Normal	Dwarf	Anther ear	Dwarf anther ear
1	7420–19	9624, S60, S136	155			
	54	9635, S81, S135	135			
	Total, 2 progenies		290			
2	7420– 5	9619, S51, S128	107	28		
	29	9628, S69, S145	53	18		
	33	9631, S74, S150	100	15		
	50	9633, S156	47	10		
	74	9640, S86, S163	67	15		
	Total, 5 progenies		374	86		
3	7420–15	9623, S57, S133	77		21	
	20	9625, S63, S139	90		28	
	35	9632, S77, S153	82		12	
	Total, 3 progenies		249		61	
4	7420– 2	9618, S48, S126	74	19	21	1
	6	9620, S52, S129	63	14	27	
	12	9622, S54, S130	63	19	18	
	21	9626, S64, S140	68	19	18	
	23	9627, S66, S142	20	6	10	
	30	9629, S70, S146	44	15	17	1
	32	9630, S71, S147	58	16	13	
	51	9634, S80, S157	67	16	16	2
	58	9636, S82, S159	75	10	11	
	61	9637, S83, S160	54	8	9	2
	62	9638, S84, S161	45	6	17	
	68	9639, S85, S162	44	7	11	2
	76	9641, S87, S164	41	12	14	
	77	9642, S88, S165	58	7	13	3
	Total, 14 progenies		774	174	215	11
5	7420–34	9757		51		
	S97–14	9775		17		6

TABLE 16

F_2 progenies of the cross dwarf × anther ear back-crossed with anther ear.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_2 PLANTS			
	F_1	F_2	Normal	Dwarf	Anther ear	Dwarf anther ear
1	7421—11	S242	11		2	
	20	S246	12		2	
	7422— 1	S247	24		3	
	3	S248	27		3	
	11	S251	22		5	
	12	S252	22		8	
	28	S255	18		7	
	32	S256	14		2	
	7423— 1	S257	23		5	
	33	S264	6		2	
	S98 — 4	9755	73		24	
	Total, 11 progenies		252		63	
2	7421—19	S245	19	1	5	
	7422— 5	S249	12	1	2	
	7	S250	17	1	2	
	26	S254	25	2	6	
	7423— 4	S258	24	4	4	
	5	S259	14	2	4	
	9	S260	25	6	3	
	18	S262	19	4	6	
	20	S263	21	2	8	
	S99 —10	9756	10	3	3	2
	Total, 10 progenies		186	26	43	2
3	7421—17	S104			9	
	21	S35, S105			13	
	7422— 6	9769, S36, S106			18	
	7423—32	9770, S47			17	
	Total, 4 progenies				57	
4	7422— 8	S37, S107			14	7
	10	S109			10	2
	16	S40, S95			52	17
	21	9768, S41, S112			37	6
	7423— 6	9771, S44			14	5
	Total, 5 progenies				127	37

TABLE 17

F_3 progenies of the cross dwarf × anther ear back-crossed with dwarf.

GROUP	PEDIGREE NUMBERS		NUMBER OF F_3 PLANTS			
	F_2	F_3	Normal	Dwarf	Anther ear	Dwarf anther ear
1	7424–13	S226	26	3		
	19	S228	24	5		
	7426– 1	S231	29	3		
	6	S233	10	3		
	7427– 2	S235	20	7		
	7428– 4	S238	30	3		
	8	S240	22	2		
	Total, 7 progenies		161	26		
2	7425– 4	S230	19	2	2	
	7426– 3	S232	16	2	3	
	8	S234	10	0	2	
	7427– 7	S236	23	1	3	
	7428– 6	S239	25	2	4	
	9	S241	22	3	5	
	Total, 6 progenies		115	10	19	0
3	7427–12	9772, S208		29		
	21	9773, S209, S210		56		
	7428–19	9774, S212		88		
	Total, 3 progenies			173		

PRODUCTION OF HIGH-PROTEIN MAIZE BY MENDELIAN METHODS[1]

H. K. HAYES

Minnesota Experiment Station, St. Paul, Minnesota

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INTRODUCTION

Some plant breeders have preferred to attack crop-improvement problems by empirical methods while others have thought it first necessary to learn the genetics of important crop characters. Thus we have such studies as the Illinois continuous-selection experiments for the purpose of producing maize races which differ in the greatest possible degree in certain chemical constituents such as high and low oil and high and low protein. Experiments of this nature show very clearly the possibilities of plant breeding. They have been widely quoted as illustrations of the creative power of selection. While they show the possibilities of selection they do not materially increase our knowledge of the genetics of maize.

An appreciation of the necessity of a knowledge of the genetics of maize led Dr. E. M. EAST to start an investigation on the physiology of maize inheritance at the CONNECTICUT EXPERIMENT STATION. The present writer had a part in these investigations during the five-year period from

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[1] One of several papers submitted to the Faculty of the Bussey Institution of HARVARD UNIVERSITY in partial fulfillment of the requirements for the degree of Doctor of Science. Published with the approval of the Director as paper No. 266 of the Journal Series of the MINNESOTA AGRICULTURAL EXPERIMENT STATION. The helpful suggestions of Dr. E. M. EAST have been of material value in the studies of inheritance of protein which are reported in this paper.

1910 to 1914, inclusive. During this period the work was carried on in coöperation with Dr. EAST. From 1915 until the present time studies in maize breeding have been carried on by the writer at the MINNESOTA EXPERIMENT STATION.

The view of learning the genetics of important economic characters and the building of improved maize varieties through the application of these principles has been the main aim at Minnesota. One phase, therefore, of the Minnesota work has been the isolation of self-fertilized lines and their recrossing.

Protein content is of much interest in the light of the Illinois studies and the writer's experience at Connecticut showed that the genetics of protein content was a complex subject. It was therefore decided to continue this investigation with Minnesota varieties. This plan was followed, not because of the belief that high-protein races were necessarily of great economic importance, but because it was thought that a knowledge of the correct genetic methods of obtaining good-yielding, high-protein races would have a direct bearing on the breeding of maize varieties which excel in other important economic characters.

REVIEW OF PREVIOUS STUDIES

A preliminary report which indicated that high-protein maize could be produced by the isolation through self-fertilization of high-protein races and their subsequent crossing was made by HAYES and GARBER (1919). The main purpose of this paper was to show that the isolation of self-fertilized lines and their subsequent crossing was a logical method for the production of improved economic varieties. Since the publication of this preliminary note, JONES (1920) has discussed the self-fertilization method of corn improvement in some detail. The views of the writer do not materially differ from those of JONES with one exception. JONES states that the recrossing of selfed lines and subsequent field selection will probably lead to the production of a variety which does not materially differ from the normal variety from which the self-fertilized lines were obtained. As all corn varieties contain many abnormalities, the elimination of these and the subsequent crossing of lines which seem of most promise seems a promising method of producing an improved variety.

EAST and JONES (1921) have recently presented an interesting paper on the genetics of protein content and have reviewed much of the literature in this field. The reader is referred to this paper and to those of JONES (1920) and HAYES and GARBER (1919) for reviews of literature in relation to the problem.

The paper by EAST and JONES (1921) will be discussed later in relation to the facts presented in this paper. The present study helps in a measure to clear up some further points in relation to EAST and JONES's discussion. Much of the data collected at Minnesota will be presented only briefly in summarized form for the purpose of showing results with a different series of varieties.

MATERIALS AND METHODS

Standard Minnesota varieties have been used in this problem. Methods of controlling pedigrees and the production of crossed and selfed seed have been carried on in much the same way as at Connecticut. For this reason it does not seem necessary to further discuss these phases.

One of the difficulties of maize breeding by artificial-pollination methods is the possibility of heavy showers which wet the tassel bags and make pollination difficult. A plan used with advantage under Minnesota conditions is to immediately replace all partially damaged bags after each rain.

Two men work together at pollination, one caring for the bag which covers the silk and the other removing the tassel bag and carefully dusting the pollen over the silk. Various men have assisted in this work: P. J. OLSON and M. N. POPE during 1915 and 1916, R. J. GARBER 1917 to 1920, and FRED GRIFFEE in 1920. The analyses have been made by the Division of Agricultural Biochemistry, the percentage figures for protein content being on a dry basis. All analyses were made in duplicate and officially recognized analytical methods were followed.

Although recognizing that the individual seed is the genetic unit and not the plant, nevertheless it was apparent that the protein content of the seeds of an ear are not greatly modified by the immediate effect of cross-pollination. The analysis of an ear represents therefore the development of protein which is possible in the light of the genetic factors carried by the mother plant and under the environmental conditions under which the plant develops.

The effect of environment and the conditions under which an artificially pollinated ear develops in relation to protein content will be first discussed.

PROTEIN CONTENT IN RELATION TO CULTURAL CONDITIONS

EAST and JONES (1921) present analyses compiled by JENKINS and WINTON showing the percentage protein content of the five subspecific maize groups. These results show that the groups do not differ very widely in average protein content. On the other hand, the range of variation for different analyses showed a content as low as 7.7 percent in one analysis of flint corn while one analysis of sweet corn showed a content as high as 17 percent. EAST and JONES compared analyses of self-fertilized upper and lower ears for six different plants. In general there was a close agreement between the protein content of the upper and lower ear of the same plant. This shows that position on the plant does not widely modify the percentage protein content.

In 1919 three normal corn varieties and one twelve-year self-fertilized strain of Red Flint were grown under different cultural conditions. In the Plant Pathology plot the environmental conditions were favorable and a heavy application of manure was made. The Plant Breeding plot appeared to lack a normal supply of food nutrients and likewise suffered from lack of moisture. Therefore the plants did not develop very vigorously and many of the leaves showed a light green color about tasseling time. Self-fertilized ears under the favorable and unfavorable conditions were analysed for protein content (see table 1).

TABLE 1

Percentage protein content under favorable and unfavorable cultural conditions.

VARIETY	PLACE GROWN	NUMBER EARS	RANGE	AVERAGE NUMBER SEEDS PER EAR	AVERAGE PROTEIN PERCENTAGE	PROTEIN P. PATHOLOGY MINUS PROTEIN P BREEDING
King Phillip	Plant Pathology	10	12.03-14.78	228	13.64	
	Plant Breeding	15	7.83-14.09	224	11.28	+2.36
Rustler	Plant Pathology	9	9.16-14.82	296	12.35	
	Plant Breeding	4	8.97-12.81	308	10.94	+1.41
Red Flint selfed 12 years	Plant Pathology	39	12.73-14.70	112	13.67	
	Plant Breeding	7	12.69-13.86	91	13.36	+0.31
Minnesota No. 13	Plant Pathology	10	10.91-14.38	471	13.08	
	Plant Breeding	5	8.75-13.75	400	11.51	+1.47

The average number of seeds per ear was about the same for the ears produced in the Plant Pathology and Plant Breeding plots. All three of the normal varieties showed much higher average percentage protein content under the favorable conditions of the Plant Pathology plot. The twelve-year self-fertilized strain of Red Flint gave about the same percentage content of protein under the two conditions.

Results in 1920 again show that protein content is very markedly influenced by cultural conditions. Ten ear samples obtained from normally pollinated Minnesota No. 13 were taken from each of two rows of Minnesota No. 13. Border rows of Minnesota No. 13 were grown which surrounded each row from which ten ear samples were taken. The ten ears for each sample were from consecutive plants in the row, the plants being spaced eighteen inches apart in the row. Results were as follows:

Row	*Protein content percentages ten-ear samples*						
96	10.02	10.19	10.54	11.25	10.62	10.65	10.78
108	12.15	11.31	11.94	11.19	11.15	11.87	11.75

It is apparent that the samples from row 108 are uniformly higher in protein content than the samples from row 96. Thus, as with most economic characters, it is impossible to determine the genetic characters of a plant without growing and examining its progeny.

TABLE 2

Correlation between percentage protein content of self-fertilized ears grown in 1919 and average percent protein in the progeny, 1920.

Average percentage of protein in the progeny 1920

1919 protein content selfed ears	8.5	9.0	9.5	10.0	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	
8.5							1		1				2
9.0					1		1	1					3
9.5							1						1
10 0								1					1
10.5							2	2					4
11.0						1	1		1		1		4
11.5								2					2
12.0				1	1	2	1			2			7
12.5	1				2			1	1				5
13.0					2			2		2			6
13.5				1	2	1	1	3			2	1	11
14.0							2	1		3	2		8
14.5									1				1
15.0				1					1			1	3
15.5										1			1
	1	0	0	3	8	4	10	13	5	8	5	2	59

$r = +.190 \pm .085$

Although it is a comparatively easy matter to isolate high-protein races through self-fertilization, there is not a very close agreement between the percentage protein content of self-fertilized ears of a variety and the protein content of the resultant progenies (see table 2).

The calculated coefficient of correlation for percentage protein content of self-fertilized ears of normal varieties and the percentage content of the progeny was +.190 ± .085. A part of the lack of relation may be due to differences in number of seeds per ear, which markedly affects the protein-content percentage. One reason for the low coefficient is probably that the conditions under which a plant develops so strongly modify the expression of its inheritance.

RELATION BETWEEN NUMBER OF SEEDS PER EAR AND PERCENTAGE PROTEIN CONTENT

A casual observation showed a strong relation between the number of seeds produced by a self-fertilized ear and percentage of protein produced by the seeds. Results for the correlation between number of seeds per ear and protein content for first-year self-fertilized ears of four normal varieties, for self-fertilized ears of two F_1 crosses between isolated self-fertilized high-protein races, for two strains self-fertilized for five years, and for two strains self-fertilized for many years, are given in tables 3 to 12 inclusive.

TABLE 3

Correlation between number of seeds per ear and protein content in first-year selfed ears of King Phillip Flint.

Percentage protein content	Number of seeds per ear 75	100	125	150	175	200	225	250	275	300	
10.5							1				1
11.0				1				1			2
11.5					1						1
12.0	1					2					3
12.5				3	2	3				1	9
13.0			1								1
13.5		1	1	3			1				6
14.0		1			1	1					3
14.5	1			1							2
15.0							1				1
15.5				1							1
	2	2	2	9	4	6	3	1	0	1	30

$r = -.303 \pm .112$

TABLE 4

Correlation between number of seeds per ear and protein content in first-year selfed ears of Mercer Flint.

Percentage protein content	Number of seeds per ear 75	125	175	225	275	325	375	425	475	
11 5					1	1				2
12.0		1		1	1					3
12.5		1		2				1	1	5
13.0	1			3	2					6
13.5		1		2		1				4
14.0		1		1						2
14.5					2					2
15.0			1		1					2
15.5										0
16.0		1								1
	1	5	1	9	7	2	0	1	1	27

$r = -.254 \pm .121$

TABLE 5

Correlation between number of seeds per ear and protein content in first-year self-fertilized ears of Minnesota No. 13.

Percentage protein content	Number of seeds per ear 75	125	175	225	275	325	375	425	475	
10 0				1						1
10.5										0
11.0										0
11.5		1								1
12.0			1		1		1			3
12.5			1		1					2
13.0			1	1	2	1				5
13.5			1	2	1	1			1	6
14.0		1	1	1		1				4
14.5		2	1	1						4
15.0	2	1	1							4
15.5		1		1						2
	2	6	7	7	5	3	1	0	1	32

$r = -.357 \pm .104$

TABLE 6

Correlation between number of seeds per ear and protein content in first-year selfed ears of Rustler Dent.

Percentage protein content	Number of seeds per ear 125	175	225	275	325	375	
10.0						2	2
11.5				1			1
12.0		1					1
12.5			3	4	2		9
13.0		3			1		4
13.5		2	1				3
14.0	1	1		1			3
14.5		1					1
15.0							0
15.5			1				1
	1	8	5	6	3	2	25

$r = -.643 \pm .104$

TABLE 7

Correlation between number of seeds per ear and protein content in Minnesota No. 13 strain, 13-46-227-1-6, which has been self-fertilized for five years.

Percentage protein content	Number of seeds per ear 75	125	175	225	
14.5				1	1
15.0			1		1
15.5			1	1	2
16.0	1	1			2
16.5		1	1		2
17.0		1	3		4
17.5	1				1
18.0		1			1
	2	4	6	2	14

$r = -.536 \pm .128$

TABLE 8

Correlation between number of seeds per ear and protein content in self-fertilized ears of related strains, 13-30-211-10-6, -10-7, -10-18, and -10-3, which have been self-fertilized for five years.

Percentage protein content	Number of seeds per ear: 125	175	225	275	325	375	425	475	525	
11 0						1				1
12.0		1								1
12.5										0
13.0				2	1	1				4
13.5				1						1
14.0		1		1	1					3
14.5		2		2		3		1		8
15.0		1	2	1	2	2		1	1	10
15.5		2		1	1	2		1		7
16.0		2	1			1	2	1		7
16 5	2	4	1	1		1	3			12
17.0		2	2			1				5
17.5		2	2	2		1				7
18.0										0
18.5		1		1						2
	2	18	8	12	5	13	5	4	1	68

$r = -.198 \pm 079$

TABLE 9

Correlation between number of seeds per ear and protein content in selfed ears of F_1 crosses, 13-30-211-13 × 13-46-226-1 and 13-30-211-7 × 13-46-226-2.

Percentage protein content	Number of seeds per ear: 25	75	125	175	225	275	325	375	425	
10.5						1				1
11.5							1			1
12.0				1						1
12.5							2		1	3
13.0									2	2
13.5						3				3
14.0						1				1
14.5				2	2	2	1			7
15.0	1		1	1	2	2		2		9
15.5	1	1		8	1	3	1			15
16.0	1	1	2	3	2	1				10
16.5		1	1							2
		3	4	15	7	13	5	2	3	55

$r = -.553 \pm .063$

TABLE 10

Correlation between number of seeds per ear and protein content in selfed ears of F_1 crosses 13-46-227-1 × 13-30-211-8, 13-46-227-2 × 13-30-211-9 and 13-30-211-9 × 13-46-227-1.

Number of seeds per ear

Percentage protein content	25	75	125	175	225	275	325	375	
12.5							1		1
13.0						1	1		2
13.5						1		1	2
14.0							1		1
14.5						1	1	1	3
15.0						2	2	1	5
15.5				1	1	3	2		7
16.0			2	2	1	3	7	1	16
16.5			3	5	2	4	1		15
17.0	2	1	2	3	1		1		10
17.5			1	1	1				3
18.0			1						1
	2	1	9	13	6	15	17	4	66

$r = -.588 \pm .054$

TABLE 11

Correlation between number of seeds per ear and protein content in self-fertilized ears of yellow dent strain, 1-6-1, etc., selfed twelve years.

Number of seeds per ear

Percentage protein conten	75	125	175	225	275	325	375	
14.0	1	1				1		3
14.5	2	1	1			1		5
15.0		2	1	2	1		1	7
15.5		1						1
16.0			1			1		2
	3	5	3	2	1	3	1	18

$r = +.228 \pm .151$

TABLE 12

Correlation between number of seeds per ear and protein content in self-fertilized ears of Red Flint strain selfed twelve years.

	Number of seeds per ear					
Percentage protein content	25	75	125	175	225	
12.5		1	1			2
13.0	2		4			6
13 5	1	7	7	2		17
14.0	1	5	7	2	2	17
14.5	2		1	1		4
	6	13	20	5	2	46

$r = +.105 \pm .098$

Summarized coefficients of correlation are given in table 13.

It is apparent from these results that protein-content percentage is very strongly modified by the number of seeds produced per ear. The correlations are uniformly large for the two F_1 crosses and consistent correlations are obtained with the normal varieties. The lack of any marked correlations for number of seeds per ear and protein content for the strains which have been selfed for many years (except for 13-46-227-1-6) are exceptions to the general rule. In this respect the results are in agreement with the effect of cultural conditions on protein content, for the protein percentages

TABLE 13

Coefficients of correlation for percentage protein content in relation to number of seeds per self-fertilized ear.[2]

STRAIN OR VARIETY	HISTORY	NUMBER EARS	CORRELATION CO-EFFICIENT
King Phillip Flint	Normal variety	30	−.303 ± .112
Mercer Flint.	Normal variety	27	−.254 ± .121
Minnesota No. 13 Dent. . . .	Normal variety	32	−.357 ± .104
Rustler Dent.	Normal variety	25	− 643 ± 104
Minnesota No. 13, selfed.	Selfed 5 years	14	−.536 ± .128
Minnesota No. 13, selfed. . .	Selfed 5 years	68	−.198 ± .079
F_1 Cross, No. 13 strains.	High × High	55	−.553 ± .063
F_1 Cross, No. 13 strains	High × High	66	−.588 ± .054
Leaming, 1−6−1, etc.	Selfed 12 years	18	+.288 ± .151
Red Flint, 69−6, etc.	Selfed 12 years	46	+.105 ± .098

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[2] Mr. A. N. WILCOX assisted in checking these correlation coefficients.

of the ears of the Red Flint strain which had been self-fertilized for twelve years were about the same for plants growing under unfavorable as under favorable conditions.

COMPARISON OF PROTEIN CONTENT OF SELF-POLLINATED AND WIND-POLLINATED MAIZE

Results obtained in the early years of study showed that self-pollinated ears were markedly higher in percentage protein content than normally pollinated ears. This was thought at first to be due largely to some change in light or temperature conditions which resulted from covering the self-fertilized ears with a paper bag. A later explanation advanced was given by EAST and JONES (1921), who believe a part of the difference in percentage protein content between self-pollinated and normally pollinated seeds is due to the immediate heterotic effect of cross-pollination. The best sort of evidence to show the immediate effect of cross-pollination is a comparison of selfed and cross-pollinated seeds of the same ear. Results of this nature are discussed in some detail by EAST and JONES who present a total of 52 analyses in which comparisons are made of self- and cross-pollinated seeds from the same ears. Averaging the results presented by EAST and JONES gives a protein content of 11.61 percent for cross-pollinated seeds and 11.76 percent for self-pollinated seeds. This is a reduction of 1.3 percent in protein content as an immediate effect of cross-pollination. Nine ears from similar pollinations, in which low and medium protein strains were used, were studied at Minnesota. The percentage protein content of the self-fertilized seeds was 15.42 and of the cross-pollinated seeds on the same ears 15.48. These differences are very small in relation to the large differences shown by EAST and JONES between open-pollinated and self-pollinated ears.

A fact of some interest not brought out by EAST and JONES would seem to show that the covering of the ear with a bag does not in itself affect the percentage protein content of the seeds produced by such a covered ear. To illustrate this point open-pollinated (O.P.) and selfed ears (S.) of the 1919 crop season are compared for protein content (see table 14).

Very marked differences in protein content for open-pollinated and self-pollinated seeds are shown for ears of F_1 crosses between high- and low-protein races, a difference of over 50 percent being obtained in one case. Of four normal varieties, three gave markedly lower protein content in open-pollinated than in self-pollinated ears. Without discussing the other results in detail we may briefly note the strains which have been selfed for several years. Of five strains of Minnesota No. 13 self-fertilized for

TABLE 14

Comparison of protein content of open-pollinated and self-fertilized ears in relation to number of years that the strain has been selfed. Grown in 1919.

VARIETY AND STRAIN NUMBER	SELFED OR OPEN-POLLINATED	NUMBER OF YEARS SELFED	NUMBER SELFED EARS	PROTEIN CONTENT	DIFFERENCE S.—O.P.	INCREASE PROTEIN CONTENT S. OVER O. P. PERCENT
High Protein × Low Protein, F_1	S.	1	6	13.62		
	O.P.	0		8.68	4.94	58.3
High Protein × Low Protein, F_1	S.	1	4	10.99		
	O. P	0		8.49	2.50	29.4
High Protein × Low Protein, F_1	S.	1	3	12.28		
	O. P.	0		8.49	3.79	44.6
Longfellow	S.	1	10	13.67		
	O. P.	0		14.11	−.44	−3.1
King Phillip	S.	1	15	12.38		
	O. P.	0		9.14	3.24	35.4
Minnesota No. 13	S.	1	6	11.51		
	O. P.	0		8.72	2.79	32.0
Rustler 31	S.	1	4	10.74		
	O. P.	0		8.95	1.79	20.0
Mercer 33–18	S.	2	12	12.68		
	O. P.	1		9.45	3.23	34.2
Mercer 33–27	S.	2	7	12.20		
	O. P.	1		10.77	1.43	13.3
King Phillip 30–26	S.	2	5	13.83		
	O. P.	1		12.50	1.33	10.6
King Phillip 30–4	S.	2	7	13.00		
	O. P.	1		11.37	1.63	14.3
Rustler 30–23	S.	2	6	13.38		
	O. P.	1		10.30	3.08	29.9
Minnesota No. 13 13–26	S.	2	7	11.67		
	O. P.	1		9.27	2.40	25.5
Minnesota No. 13 13-3	S.	2	3	13.01		
	O. P.	1		10.55	2.46	23.3

TABLE 14 (continued)

VARIETY AND STRAIN NUMBER	SELFED OR OPEN-POLLINATED	NUMBER OF YEARS SELFED	NUMBER SELFED EARS	PROTEIN CONTENT	DIFFERENCE S. — O.P.	INCREASE PROTEIN CONTENT S. OVER O. P. PERCENT
Minnesota No. 13	S.	2	10	13.29		
13-22	O. P.	1		11.99	1.30	10.8
Minnesota No. 13	S.	2	2	12.19		
13-23	O. P.	1		12.18	.01	0.0
Minnesota No. 13	S.	2	3	13.01		
13-24	O. P.	1		10.13	2.88	28.4
Minnesota No. 13	S.	5	14	16.39		
13-46-227-1-6	O. P.	4		14.94	1.45	9.7
Minnesota No. 13	S.	5	14	15.45		
13-30-211-10-3	O. P.	4		15.87	−.43	−2.6
Minnesota No. 13	S.	5	18	15.52		
13-30-211-10-18	O. P.	4		15.15	.37	0.0
Minnesota No. 13	S.	5	20	15.45		
13-30-211-10-6	O. P.	4		16.74	−1.29	−7.7
Red Flint	S	12	7	13.36		
	O. P.	11		13.68	−.32	−2.3

five years, three gave a higher protein content in open-pollinated than in self-pollinated ears. Likewise the twelve-year self-fertilized strain of Red Flint gave a higher protein content in the open-pollinated ears though the differences were very small. These facts seem to show that covering an ear with a bag does not in itself greatly modify protein content.

The facts presented here and the data of EAST and JONES lead to the conclusion that the main cause of the difference in protein content of self-fertilized and normally pollinated ears is the marked correlation between the percentage protein content of an ear and the number of seeds produced by the ear. This fact deserves careful consideration in an analysis of the effects of continuous selection in self-fertilized lines. On the average there is a gradual decrease in yield in self-fertilized lines of maize which for a considerable number of years or until homozygosis is reached is roughly proportional to the number of years which the strain has been self-fertilized. During the period of this reduction in yield there is a corresponding reduction in the average number of seeds produced by either self-pollinated or normally pollinated ears. This leads naturally, when one considers the correlations previously noted, to a gradual increase in percentage protein content.

INHERITANCE OF PROTEIN CONTENT

It has been noted previously that the percentage protein content of the seeds of an F_1 ear frequently closely corresponds to the percentage protein content of the lower-protein parent. It was also demonstrated by HAYES and GARBER (1919) that F_1 ears of crosses between self-fertilized high-protein races have considerably lower percentage protein content than the parental self-fertilized lines. These facts and a considerable body of similar evidence presented by EAST and JONES led these investigators to conclude that although "there was a semblance of dominance of low protein" over high protein, this was largely due to the effect of heterosis. This apparent dominance has, however, much value from the breeding standpoint. Whether the apparent recessiveness of high protein is due to genetic factors for protein content or partly due to the effect of other growth factors does not detract from the value of the relation between protein content of a self-fertilized ear and the protein content of its progeny. As high protein apparently behaves in inheritance as a recessive character, all that is necessary in order to isolate high-protein races is to practice self-fertilization and then determine which lines breed true for higher protein content by an analysis of the average of the progeny. Even though continued selection isolates higher-protein races, this may not always be due to the isolation of genetic factors for a greater synthesis of protein but due to the elimination of growth factors and the consequent reduction in size of ear and seed which results in a higher percentage protein content.

Crosses between high- and medium-protein races which had been isolated through self-fertilization were made at Minnesota in 1918. A considerable number of F_2 ears were grown in 1920 and normal pollination was permitted. The percentage protein content of the F_2-generation ears was compared with the content of ears of Minnesota No. 13 and with self-fertilized ears of the long-time-selfed strain of Red Flint which was grown in 1919 (see table 15).

The F_2 generation was about as variable for percentage protein content as normal ears of Minnesota No. 13. The low coefficient of variability of the Red Flint ears is of considerable interest. Comparatively homozygous lines for protein content may therefore be isolated, since segregation does occur in those generations which are commonly known as the segregating generations. Protein content is therefore inherited in much the same way as other characters which are dependent for their full expression on many different inherited factors of the plant and likewise upon environmental conditions.

TABLE 15

Comparison of variability for percentage protein content of normally pollinated Minnesota No. 13 ears, F_2 open-pollinated ears of a cross of Minnesota No. 13 high-protein strain × Minnesota No. 13 medium-protein strain, and self-fertilized ears of a twelve-year self-fertilized strain of Red Flint (1920).

SOURCE	PROTEIN PERCENTAGE CLASSES												TOTAL	MEAN	C. V.
	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0	15.5	16.0			
Normal Minnesota No. 13	7	8	12	19	18	10	12	2					88	12.2±.07	7.46±.38
F_2 cross..................		2	3	9	12	21	16	11	7	4	2	1	88	13.3±.07	7.59±.39
12-year self-fertilized Red Flint..................					2	6	17	17	4				46	13.7±.05	3.50+.25

ISOLATION OF HIGH-PROTEIN RACES THROUGH SELF-FERTILIZATION

EAST and JONES have given considerable evidence to show the ease with which high-protein races may be isolated through self-fertilization. For this reason Minnesota data will be only briefly noted. The facts are presented solely for the purpose of showing that high-protein races may be isolated in standard Minnesota varieties (see table 16).

It hardly seems necessary to emphasize these results by extended discussion. The results corroborate those of EAST and JONES and show the value of the method. Perhaps it may serve a useful purpose to point out that similar methods seem the logical means of isolating self-fertilized families which are resistant to plant diseases such as corn smut and root rot. Chlorophyll degeneracies which are prevalent in nearly all commercial varieties may be eliminated by similar means. The idea deserves wide consideration in the hands of plant breeders interested in maize. If all experimental stations in the corn belt could vigorously attack the problem and if coöperation between investigators could be more fully developed the results would be of undoubted value to the corn-growing industry.

THE USE OF SELF-FERTILIZED LINES IN MAIZE IMPROVEMENT

Several methods of the utilization of self-fertilized lines suggest themselves. JONES'S (1920) suggestion of the double cross seems worthy of extended trial. The practical difficulties of the method, while not so great as the use of F_1 crossed seed of two self-fertilized lines (a method originally outlined by SHULL), are due to the necessity of producing four strains annually and the making of three crosses before obtaining seed for commercial planting.

TABLE 16

Isolation of high-protein strains by self-fertilization.

STRAIN NUMBER	NUMBER YEARS SELFED	YEAR GROWN	PROTEIN CONTENT OF PARENT	PROTEIN CONTENT BULK ANALYSIS OF PROGENY
Minnesota No. 13 13-30	1	1916	16.52	14.47
Minnesota No. 13 13-46	1	1916	16.66	15.82
Minnesota No. 13 13-12	1	1916	16.99	14.10
Minnesota No. 13				
13-30-211	2	1917	16.90	13.06
13-46-226	2	1917	——	12.44
13-46-227	2	1917	17.16	14.03
13-12-181	2	1917	17.40	10.87
Minnesota No. 13				
13-30-211-10	3	1918	——	14.88
13-46-227-1	3	1918	15.10	15.31
13-46-227-3	3	1918	12.72	14.88
13-12-181-2	3	1918	15.28	15.81
Minnesota No. 13				
13-30-211-10-6	4	1919	17.19	15.88
13-30-211-10-14	4	1919	17.19	15.45
13-30-211-10-18	4	1919	17.69	15.15
13-30-211-10-3	4	1919	16.50	15.87
13-46-227-3-6	4	1919	16.19	14.94
13-46-227-1-8	4	1919	15.31	——
13-12-181-2-1	4	1919	15.69	——
Minnesota No. 13				
13-46-227-3-6-26	5	1920	15.59	17.87
13-46-227-3-6-20	5	1920	16.50	17.62
13-30-211-10-6-6	5	1920	16.73	18.12
13-30-211-10-6-10	5	1920	16.57	16.59
13-30-211-10-14-9	5	1920	17.16	17.90
13-30-211-10-14-13	5	1920	18.43	18.22
13-30-211-10-18-22	5	1920	15.20	17.46
13-30-211-10-18-25	5	1920	17.47	16.90
13-12-181-2-1-1	5	1920	14.63	16.44
13-12-181-2-1-9	5	1920	16.72	16.45
13-12-181-2-1-14	5	1920	14.81	18.00
Rustler 31-26-6	2	1920	14.70	17.22
King Phillip 30-4-3	2	1920	13.91	17.50
Mercer 33-27-1	2	1920	15.00	15.03

EAST and JONES point out a logical means of improving strains and show that for high protein the method has proven valuable. This is the isolation of high-protein races, the cross of these races and the subsequent selection in the segregating generations of those self-fertilized ears which excel for protein content. This is the Mendelian plan which has been adopted by several breeding centers as a means of increasing the yield or improving the economic characters of self-fertilized plants such as our small grain crops.

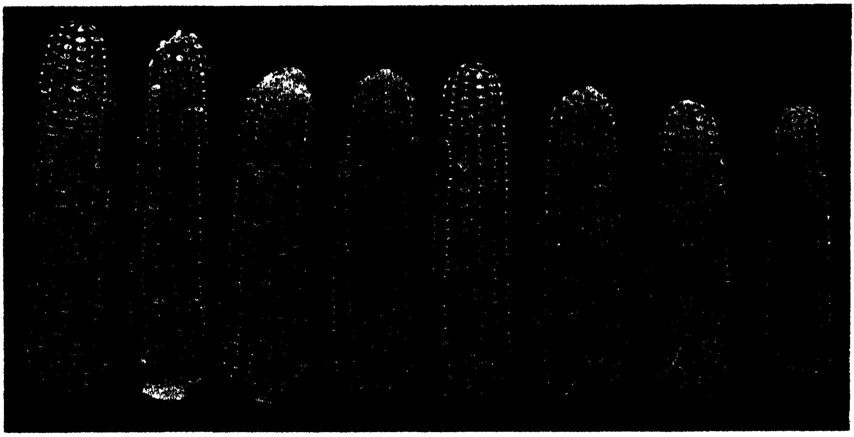

FIGURE 1.—Average ears of Minnesota No. 13 grown in comparison with the double cross of four high-protein strains. 1920. (Photo by HORTON.)

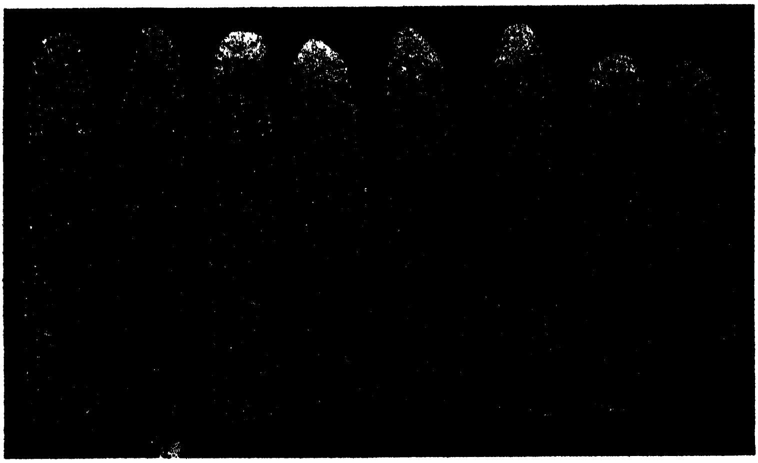

FIGURE 2.—Double cross of four high-protein self-fertilized strains. Same focus as above ears of Minnesota No. 13. 1920. (Photo by HORTON.)

Another method deserves trial by maize breeders. After several strains have been isolated they may be crossed and field selection practiced for desirable stalks and ears. Such a synthetically produced variety should in some cases, depending on the value of the combination obtained from crossing the selfed strains, give a variety of maize which is a great improvement over the normal variety from which the selfed strains were originally obtained.

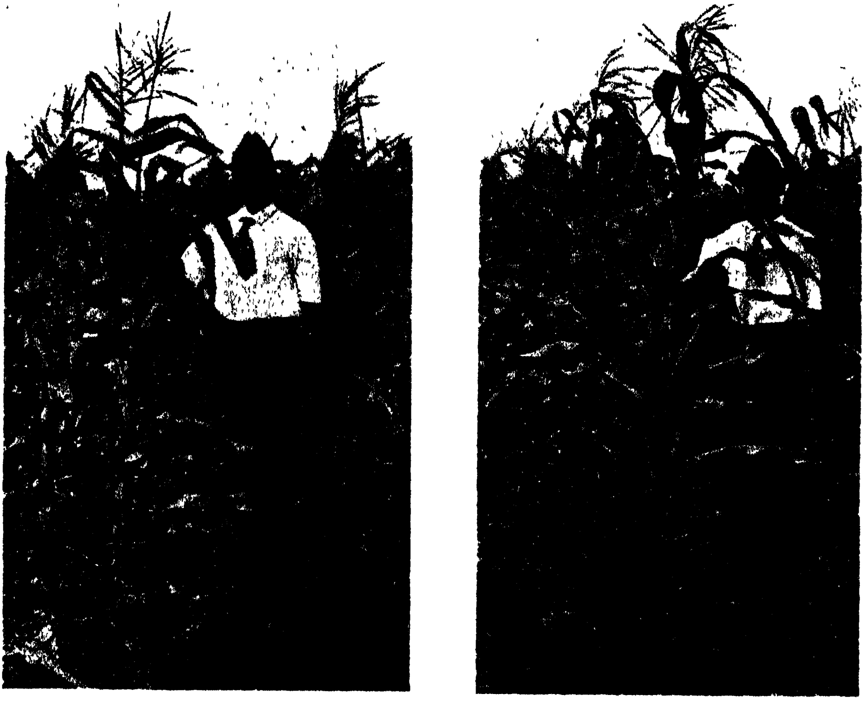

FIGURE 3.—Comparative vigor of Minnesota No. 13, left, and the double cross of four self-fertilized high-protein strains at the right. 1920. (Photo by HORTON.)

A double cross of four high-protein strains was tested in 1920. One of the original crosses was made by JONES of Connecticut who used a high-protein strain of Leaming and one of Illinois High Protein. The cross between two Minnesota No. 13 high-protein strains was crossed with the F_1 cross made by JONES and the double cross grown in replicated plot tests in 1920 and compared with Minnesota No. 13. The double cross was slightly later than Minnesota No. 13 but it produced ears very freely and looked very promising. Due to dry weather late in the season the Minnesota No. 13 filled somewhat better than the double cross. Comparative yields and protein content of Minnesota No. 13 and of the double cross are given in table 17 (see figures 1 to 3).

TABLE 17

Comparison of protein content, yields and shelling percent of Minnesota No. 13 and reciprocal double crosses.

SOURCE	SHELLED CORN PER STALK GRAMS	SHELLING PERCENT	PERCENTAGE PROTEIN CONTENT
Double cross	128.2	77.0	14.92
Reciprocal	141.9	76.0	15.14
Minnesota No. 13	144.9	80.6	12.37

An attempt was started in 1916 to produce synthetically a high-protein variety of corn by crossing isolated high-protein races of Minnesota No. 13.

FIGURE 4.—Average ears of Minnesota No. 13. Isolated pasture plot. 1920. (Photo by HORTON.)

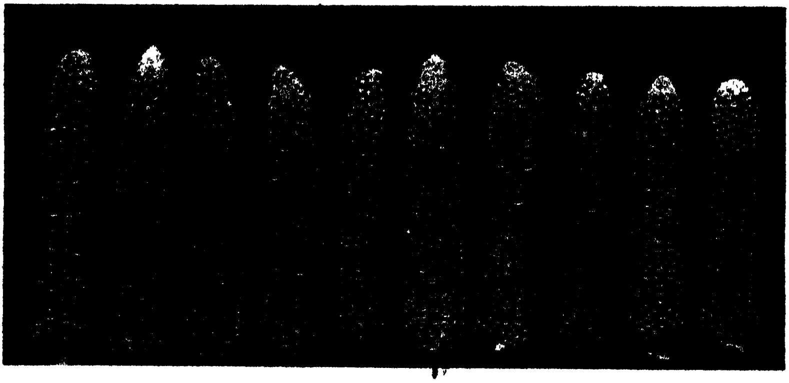

FIGURE 5.—Average ears of High Protein Minnesota No. 13. Produced by crossing two high protein strains and subsequent selection. Isolated pasture plot, 1920. Same reduction as figure 1. (Photo by HORTON.)

The original cross was made between two apparently high-protein strains. In the F_2 generation, however, lethal white chlorophyll seedlings were obtained. F_3 lines were grown from self-fertilized F_2 ears and reciprocal crosses within a line and between different lines were made. Only those families were used which were homozygous for an absence of the white-seedling degeneracy. Seeds of those F_3 reciprocal crossed ears which showed high protein content in both parents, were mixed together and used to plant an isolated plot. A determination of the yield and protein content of this isolated plot was made and compared with a similar plot of Minnesota No. 13 (see table 18 and figures 4 and 5).

TABLE 18

Comparison of yields and protein content of normal Minnesota No. 13 and a synthetically produced Minnesota No. 13 obtained by crossing high-protein strains.

SOURCE	YIELD EAR-CORN PER STALK, GRAMS	PROTEIN CONTENT PERCENTAGE
Normal Minnesota No. 13......	122.6	12.01
High Protein No. 13...........	122.6	14.99

The results indicate the value of the method. These studies have led to the attempt to breed maize at Minnesota for economic characters other than protein by an adoption of a Mendelian plan which, except for the details of the work, is not essentially different from the method which is being used for small grains and which has already proved its value through actual test.

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CORRELATION, CAUSATION AND WRIGHT'S THEORY OF "PATH COEFFICIENTS"[1]

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INTRODUCTION

What occasions a result? What is its determining cause?

We have an answer to questions of this sort in many specific cases, but none of the attempts to produce a general formula universally applicable for the solution of such questions has been entirely satisfactory. The present paper is a critical discussion of the latest solution offered, the method of "path coefficients" as proposed by WRIGHT (1921 a).

The conscious attempts to obtain a mathematical measure of causation, or to establish a mathematical criterion by which to test the truth of the statement that one event is the cause of another, have been, in the main, recent developments, but they are all essentially refinements of the simple method of concluding, because the observer has never known one event to happen without being followed by the other, that the two are therefore cause and effect. Although there may possibly be a few cases that appear to be exceptions when the observer is forming his conclusions, he is apt to reject them as being due to certain factors which he overlooked. This whole procedure is simply a non-mathematical way of determining roughly the degree of association or of correlation between the two events and regarding a high correlation either as causation itself or as evidence of a

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[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, JOHNS HOPKINS UNIVERSITY, No. 42.

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"causal relation." GALTON (1889) says in regard to correlation between organs:

> "It is easy to see that correlation must be the consequence of the variations of the two organs being partly due to common causes. If they were wholly due to common causes, the co-relation would be nil. Between these two extremes are an endless number of intermediate cases."

This is the opinion of the man who appears to have been the first to conceive the idea of mathematical correlation (PEARSON 1920). The works of BRAVAIS and of GAUSS are treatments of the probability of errors of observation, and afford no basis for a claim that either of them discovered correlation.

"Causation" has been popularly used to express the condition of association, when applied to natural phenomena. There is no philosophical basis for giving it a wider meaning than partial or absolute association. In no case has it been proved that there is an inherent necessity in the laws of nature. Causation is correlation.

In his "Grammar of Science," PEARSON (1900), who developed the product-sum correlation coefficient now used, says in regard to scientific law and causation:

> "Law in the scientific sense only describes in mental shorthand the sequences of our perceptions. It does not explain *why* those perceptions have a certain order, nor *why* that order repeats itself; the law discovered by science introduces no element of necessity into the consequences of our sense impressions; it merely gives a concise statement of *how* changes are taking place. That a certain sequence has occurred and recurred in the past is a matter of experience to which we give expression in the concept *causation*; that it will continue to occur in the future is a matter of belief to which we give expression in *probability*. Science in no case can demonstrate any inherent necessity in a sequence, nor prove with absolute certainty that it must be repeated."

. . . . . .

> "When we say that we have reached a 'mechanical explanation' of any phenomenon, we only mean that we have described in the concise language of mechanics a certain routine of perceptions. We are neither able to explain why sense-impressions have a definite sequence, nor to assert that there is really an element of necessity in the phenomenon. Regarded from this standpoint, the laws of mechanics are seen to be essentially an intellectual product, and it appears absolutely unreasonable to contrast the mechanical with the intellectual when once these words are grasped in their accurate scientific sense."

> "No phenomenon or stage in a sequence has only one cause, all antecedent stages are successive causes, and, as science has no reason to infer a first cause, the succession of causes can be carried back to the limit of existing knowledge and beyond that *ad infinitum* in the field of conceivable knowledge. When we scientifically state causes we are really describing the successive

stages of a routine of experience. 'Causation' says JOHN STUART MILL 'is uniform antecedence' and this definition is perfectly in accord with the scientific concept."

"The causes of any *individual* thing thus widen out into the unmanageable history of the universe. The causes of any finite portions of the universe lead us irresistibly to the history of the universe as a whole."

The above quotations are made, not as an appeal to authority, but because Professor PEARSON has already inimitably summarized the subject.

The theory of planetary motion is an intellectual concept that has been built up to describe, at least approximately, the observed events of the movements of the planets. If the theory holds, certain things *must* of logical necessity be true, but we must beware lest we unconsciously and illogically think that the necessity that lies in the concept also inheres in the order of nature which the concept attempts to describe.

The idea of determination, in the sense of causes fixing beforehand the nature of the effect, is based upon the belief in an inherent necessity in the order of things. We have seen that no such necessity can be proved. Therefore, determination should be used only in the sense of an ability to predict with fair accuracy the value of an effect when the values of its principal causes are known. This ability is based upon our knowledge of the degree of association between the causes and the effect.

To contrast "causation" and "correlation" is unwarranted because causation is simply perfect correlation. Incomplete correlation denotes partial causation, the effect here being brought about by more than one important cause. Many things show either high or perfect correlation that, on common-sense grounds, can not possibly be cause and effect. But we can not tell *a priori* what things are cause and effect and a conflict between our observations and our "common-sense" belief may be due either to an unwarranted belief or else to the calculation of our coefficients of correlation from too few data.

If Röntgen rays be directed against a brick wall, one can see through it. But it would indeed be difficult to imagine two more dissimilar things than Röntgen rays and sight through a brick wall; and yet, because these are invariably correlated, they are now so accepted. An example of high correlation and no causal relation might be the correlation over a two year period between the weight of a child born in 1917 and the tonnage production of ships in the United States. Here the data are evidently insufficient. We know that children born in 1917 grew at practically the same rate as children have always grown, but that ships were produced at a much faster rate in order to meet the war needs. Therefore if we correlate the weight of children for their first two years of life with the tonnage production of ships over any long period we may be sure that the correlation coefficient would be practically zero.

It seems clear that perfect correlation, *when based upon sufficient experience,* is causation in the scientific sense.

THE METHOD OF PATH COEFFICIENTS

This method is claimed by WRIGHT (1921 a, b) to provide a measure of the influence of each cause upon the effect. Not only does it enable one to determine the effects of different systems of breeding, but provides a solution to the important problem of the relative influence of heredity and environment. To find flaws in a method that would be of such great value to science if only it were valid is certainly disappointing. The basic fallacy of the method appears to be the assumption that it is possible to set up *a priori* a comparatively simple graphic system which will truly represent the lines of action of several variables upon each other, and upon a common result.

In his introduction WRIGHT (1921 a) states:

> "The method depends on the combination of knowledge of the degrees of correlation among variables in a system with such knowledge as may be possessed of the causal relations. In cases in which the causal relations are uncertain the method can be used to find the logical consequences of any particular hypothesis in regard to them."

We have to set up a graphic system of the way we think the variables act upon each other. If we have enough observed correlation coefficients, we calculate the path coefficients and the coefficients of determination. The results are then compared with what we expect or have observed to be true in nature, and if they are in pretty close agreement our hypothesis is accepted, and we are to regard the system as showing the true relations between variables. If they are not in agreement, the hypothesis upon which we built our system must be wrong and a new one will have to be tried. But even if the observed and calculated values of the correlation coefficients agree, we can by no means be sure that we have set up the true system. An infinitude of values of x and y satisfy the simple equation $x^2+y^2=1$. The arrangement of the system depends entirely upon the judgment of the observer, and no *test* of that judgment follows in the least.

In all set-ups, or diagrams of systems, it is necessary to cut off the lines of causation at points not very far back in the chain of causes. This leaves two or more cause groups with nothing behind them, although it is inconceivable that these groups have not some common causes. If we put into our system all important causes we know of, and all the important causes of these, and so on back, we would cover the whole universe and even then find no logical stopping place. There is absolutely nothing in the method to tell us how far back we should go. Apparently WRIGHT himself goes back as far as the observed correlation coefficients, which are needed to solve the equations, will permit. But extension backwards will change the values of path coefficients and coefficients of determination, and may also render the whole system unsolvable.

Two methods for the solution of the hypothetical systems are given; the direct, using determinants; and the indirect, using simultaneous equations. The indirect method is said to be less laborious than the direct, and this method "is more flexible in that it can be used to test out the consequences of any assumed relation among factors." (WRIGHT 1921 a, p. 578). Also (loc. cit., page 579):

> "One should not attempt to apply in general a causal interpretation to solutions by the direct methods. In these cases, determination can usually be used only in the sense in which it can be said that knowledge of the effect determines the probable value of the cause. This is the sense in which PEARSON's formula for multiple regression must be interpreted."

Measures of association or correlation are provided by mathematics, but we have no mathematical test which will enable us to tell absolutely whether or not to interpret any particular case as one of causation. We can not be sure that we have taken enough cases or a sufficiently large number of variables into consideration. By using the correlation coefficient we *know* that, in the sample of the universe which we have studied, certain variables were more or less closely associated than others, as indicated by the value of r, the coefficient of correlation, provided that the variations of each variable followed the Gaussian, or normal, curve of error. From this knowledge we are led to *believe*, either that the sample tried is not a fair one and another one is needed, or that certain causal relations probably do or do not exist. Statistical methods, particularly multiple correlation, indicate causes when they are used with common sense and upon the data of critical experiments. But the method of path coefficients does not aid us because of the following three fallacies that appear to vitiate this theory. These are (1) the assumption that a correct system of the action of the variables upon each other can be set up from *a priori* knowledge; (2) the idea that causation implies an inherently necessary connection between things, or that in some other way it differs from correlation; (3) the necessity of breaking off the chain of causes at some comparatively near finite point. The applications of this theory in the latter part of this paper give impossible results and illustrate faults in the method.

THE MATHEMATICS OF PATH COEFFICIENTS

The section on Definitions in WRIGHT's paper opens with the following sentences (italics mine):

> "We will start with the assumption that the direct influence along a given path can be measured by the standard deviation remaining in the effect after all other possible paths of influence are eliminated, *while variation of the causes back of the given path is kept as great as ever, regardless of their relations to the other variables which have been made constant.* Let X be the dependent variable or effect and A the independent variable or cause. The expression $\sigma_{X.A}$ will be used for the standard deviation of X, which is found under the foregoing conditions, and may be read as the standard deviation of X due to A."

If X is regarded as being completely determined by A and B, WRIGHT's $\sigma_{X.A}$ is somewhat like YULE's $\sigma_{X.B}$, the standard deviation of X when B is held constant; and when X is completely determined by A, B and C, it is somewhat like YULE's $\sigma_{X.BC}$. The physical interpretation of $\sigma_{X.B}$ and $\sigma_{X BC}$ is very easy. If from a large group, all the cases having the same-size B's, or the same-size B's and C's, were picked and the standard deviation of the X's in this new group was found, the result would be the familiar $\sigma_{X.B}$ or $\sigma_{X.BC}$. But to make this correspond to WRIGHT's $\sigma_{X.A}$ we should in some manner have to keep all the variables back of B, which affected A, *just as variable as before.* How this might be done is difficult for the mind to grasp.

In figure 1, if we wish to get WRIGHT's $\sigma_{X.A}$ we must not let any action come along the path BX but must make as much as before come along $A\ X$, $A\ D$ and $A\ C$. If A and B are correlated, and if B is given a constant value it follows that the variation in C must be reduced and the path coefficient along the line $A\ C$ changed. In holding constant a variable we are really picking out only observations of it that have the same value and are considering the causes and effects of this new group. The results of a constant effect are obviously less variable than those of a variable effect; and, although there may conceivably be some compensatory changes in the causes, it seems impossible that they should vary as much in producing a constant effect as in producing a variable one. This simple point of logic WRIGHT appears to have overlooked.

In a paper in Genetics, WRIGHT (1921 b) says:

> "A path coefficient differs from a coefficient of correlation in having direction. The correlation between two variables can be shown to equal the sum of the products of the chains of path coefficients along all the paths by which the variables are connected."

The pure mathematics by which this is shown is apparently faultless in the sense of mere algebraic manipulation, but it is based upon assumptions which are wholly without warrant from the standpoint of concrete, phenomenal actuality.

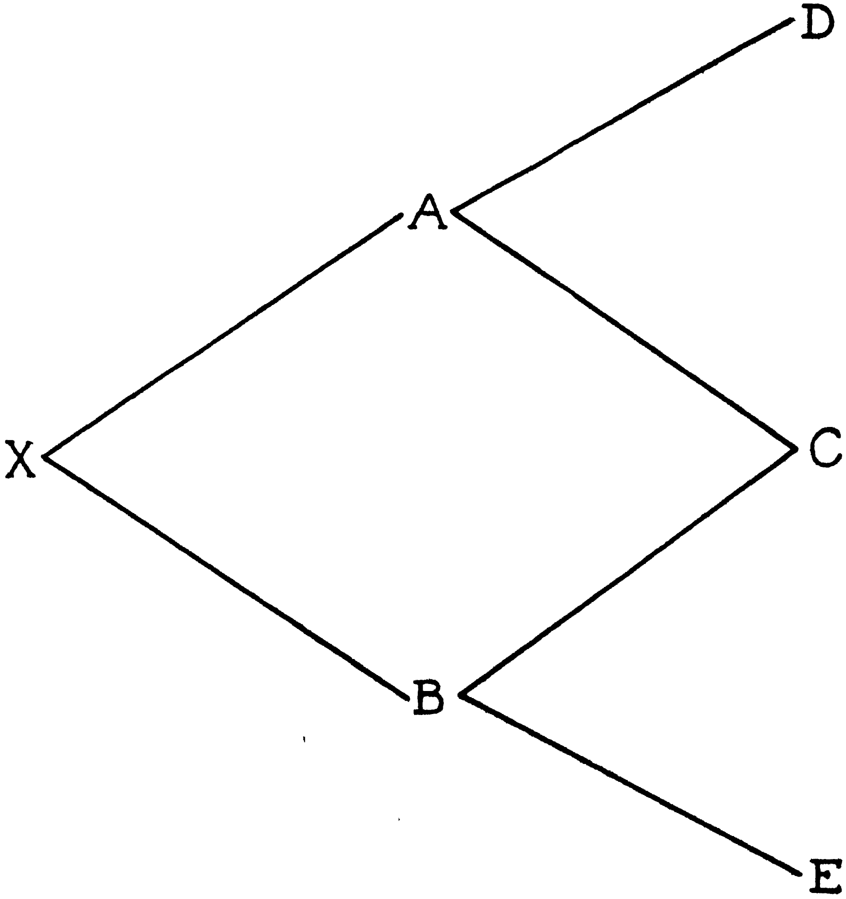

FIGURE 1.—An effect, X, determined by two correlated causes.

WRIGHT'S GUINEA-PIG EXAMPLE

The guinea-pig is "intended merely to furnish a simple illustration of the method" (WRIGHT 1921 a, p. 570). It shows us how to measure the relative importance in determining the birth weight of guinea-pigs, X, of the factors Q, prenatal growth curve; P, gestation period; L, size of litter; A, heredity and environmental factors which determine Q apart from size of litter; C, factors determining gestation period apart from size of litter. The "prenatal growth curve" is apparently the average growth per unit of time during the gestation period. If we multiply the average growth by the time we necessarily get the birth weight, but it is impossible to get the average growth until the total growth and the time are known. If $\frac{X}{P} = Q$, then any two of the variables mathematically determine the third. It is as logical to say that the birth weight and the gestation period determine the prenatal growth curve, as to say that the gestation period and the prenatal growth curve determine the birth weight.

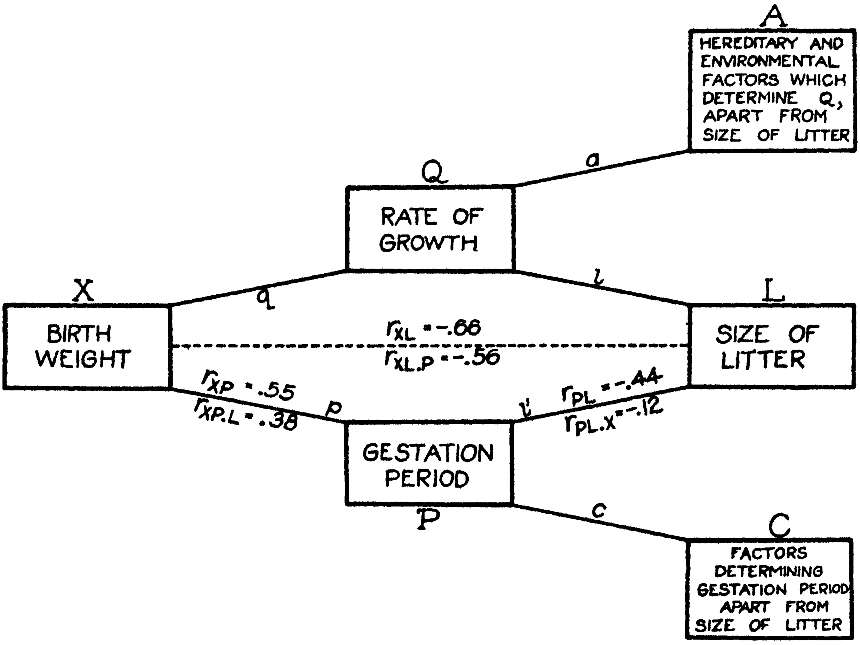

FIGURE 2.—The system set up by WRIGHT (1921 a) for finding the relative importance of factors determining the birth weight of guinea-pigs.

In solving the guinea-pig problem three things are known from experience (figure 2). These are the correlations between birth weight and interval between litters, which is assumed to be the gestation period if less than 75 days, $r_{XP} = +.5547$; birth weight and size of litter, $r_{XL} = -.6578$; and between gestation period and size of litter, $r_{PL} = .4444$. These are the realities. From the general equation where the coefficient of correlation is the sum of the products of the path coefficients along all the chains of causes connecting the two variables, he derives three equations:

$$(1)\ r_{XP}=p+q\,l\,l'$$
$$(2)\ r_{XL}=q\,l+p\,l'$$
$$(3)\ r_{PL}=l'$$

We might get equations (1) and (2) directly from figure 2, but to be consistent throughout we must get equation (1) from the system shown in figure 3, equation (2) from figure 4, and equation (3) from figure 5. In each of these systems one of the variables is made a cause of itself with a path coefficient of unity between variable as cause and variable as effect.

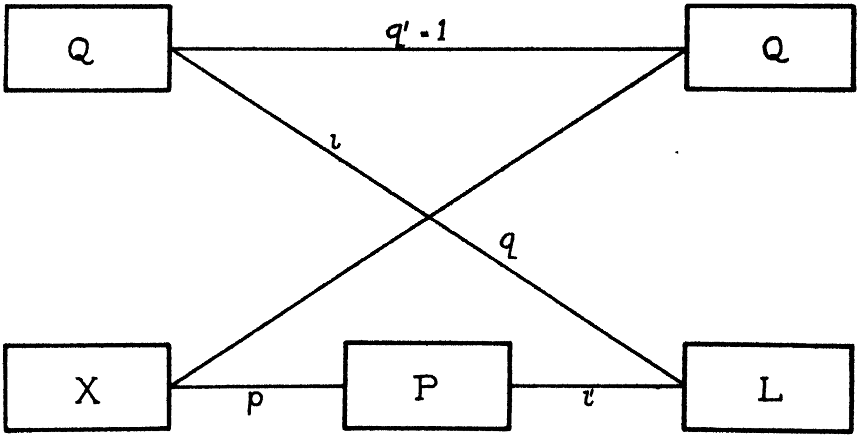

FIGURE 3.—System from which equation (1) would be obtained.

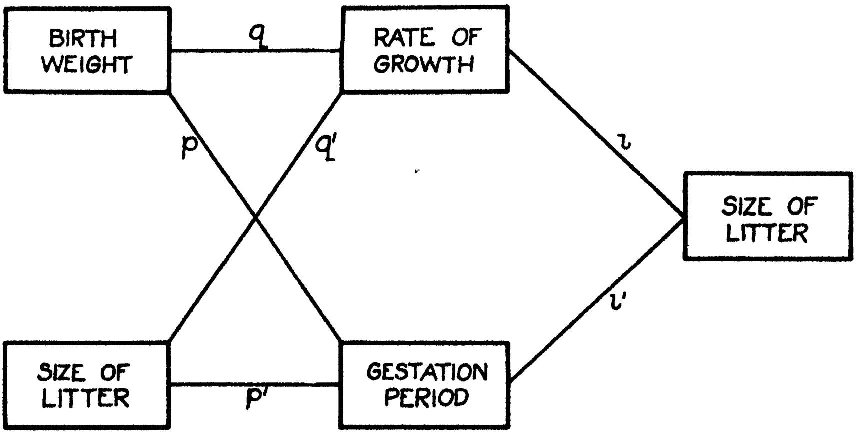

FIGURE 4.—System from which equation (2) would be obtained. $q'l=1$, and $p'l'=1$.

This seems rather forced, but if we attempt to obtain the relation from the original diagram, what is there to prevent our setting $r_{PL}=l'+pq\,l$; that is, following all the possible paths of the original set-up in getting equation (3)? Perhaps in tracing the chains of causes we are not allowed to come to the effect through something that follows it. Such a rule would be meaningless when the true relation of cause and effect, that of invariable association, is kept in mind.

Three more equations are based upon the fact that the sum of the coefficients of determination of any effect must be equal to unity. These coefficients are simply the squares of the path coefficients between cause and effect; except when there are correlated causes, when there is also a coefficient of determination which represents the action of the correlated causes taken together. This coefficient is twice the product of the path coefficients from each cause to the effect, times the coefficient of correlation between the causes. The additional equations in this case are

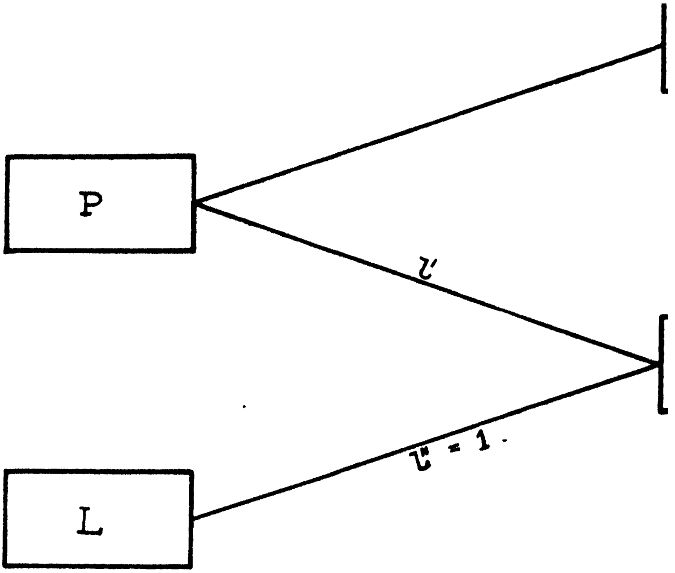

FIGURE 5.—System from which equation (3) would be obtained.

$$(4)\quad q^2+p^2+2q\,p\,l\,l'=1$$
$$(5)\quad a^2+l^2=1$$
$$(6)\quad l'^2+c^2=1$$

The values obtained from the six equations are assumed to be measures of realities if the diagrams accurately represent the causal relations. Figure 6 shows the values obtained for each path coefficient. No value of the probable error of any constant is given by WRIGHT. If the method of path coefficients were valid, a knowledge of the probable error of any constant would be essential in many cases. The correlation between size of litter and gestation period for constant birth weight, using only the observed r's the writer computed and found to be $r_{PL.X} = -.12$. How are we to account for the difference between this value and $r_{PL} = l' = -.44$? The $r_{PL.X}$ means that when we select groups of guinea-pigs of the same birth weight the correlation between size of litter and gestation period is greatly reduced, and the path coefficient and coefficient of determination are correspondingly reduced, the latter taking the value $-.014$. Therefore, when guinea-pigs of equal birth weight are considered, the size of the litter has practically no effect upon the gestation period.

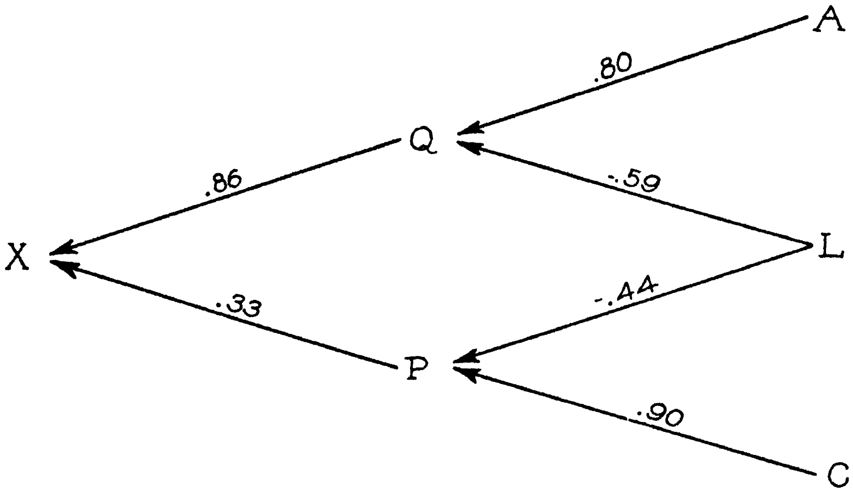

FIGURE 6.—Showing the values obtained by WRIGHT (1921 a) for the path coefficients between the factors determining birth weight of guinea-pigs. See figure 2.

TEST OF WRIGHT'S METHOD

Except in unusual cases we can check the results of this method of WRIGHT'S only by testing them with what we *think* on common sense grounds ought to be true. In the hands of a man well acquainted with the realities in the field he is investigating, this method would be likely to lead to results not far from the truth, because if any values appear to be inconsistent, a new set-up of causes and effects will be made. Guesses by a trained man would be on the whole quite as good and much less work; whereas an untrained man can not be sure of the validity of his results at all, because he is not familiar with the realities in the field of study.

Let us attempt to apply this method to two examples where we know more of the correlation coefficients, and can get our path coefficients and coefficients of determination in more than one way.

Example 1

We are interested here in the relative part played by the number of seeds per pod, and the number of ovules per pod, in determining the seed weight of the seeds produced. We set up the diagram shown in figure 7, making (1) seed weight, be determined by (2) seeds per pod, (3) ovules per pod, and (4) other causes than (2) and (3). Seeds per pod is determined by (5) pods per plant and (6) other causes than (5) which affect (2). Ovules per pod is also determined by (5) and by (7) a group of other causes. Behind these we do not go as we have not enough observations to solve a more complicated system and we will assume that we are to be satisfied with approximate results. This figure is identical with the diagram (figure 2) used by WRIGHT (1921 a) in setting up his equations, except that we have an all-other-causes path affecting (1) directly. This enters only in the equation which makes the summation of the coefficients of de-

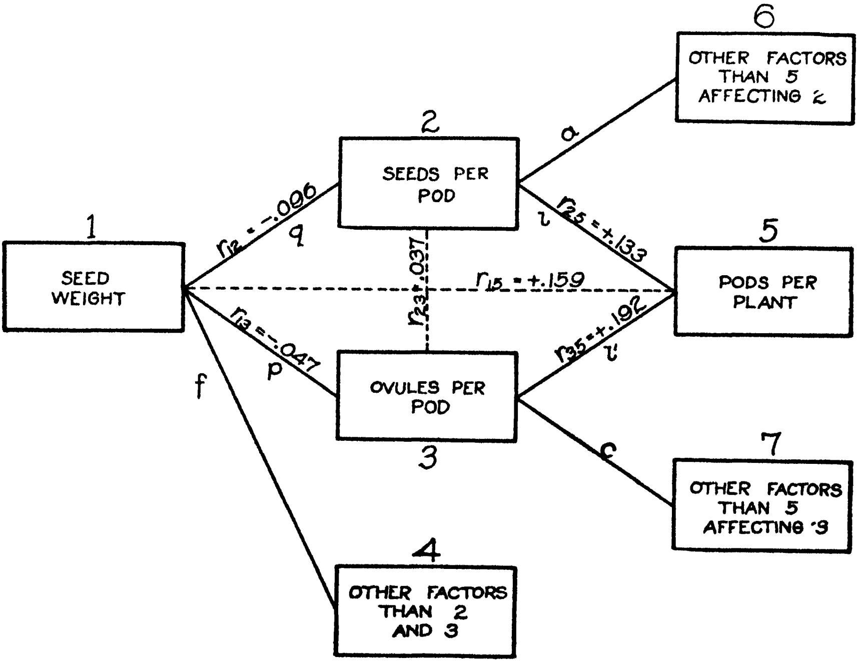

FIGURE 7.—The system set up for example 1, showing the observed correlation coefficients.

termination equal to unity. The correlation coefficients shown in the figure are from J. ARTHUR HARRIS (1913 a, 1913 b, 1916) and although of low absolute values, they are very probably significant, because the probable errors where given are extremely small, and all the constants appear to have been based upon a very wide experience.

The equations involving only the first powers of the path coefficients are:

$$\begin{aligned}&(1)\ r_{13}=p+q\,l\,l'\\&(1\text{ bis})\ r_{12}=q+p\,l\,l'\\&(2)\ r_{15}=q\,l+p\,l'\\&(3)\ r_{25}=l\\&(4)\ r_{35}=l'\end{aligned}$$

Taking r_{13}, r_{15}, r_{25}, and r_{35} as known, we will use the above equations to get the path coefficients and the coefficients of determination.

Substituting the numerical values of r_{13}, l and l' in equation (1) we have

$$\begin{aligned}p&=-.047-(.133\times.192)\,q\\&=-.047-.025536\,q.\end{aligned}$$

Equation (2) now becomes

$$\begin{aligned}.159&=.133\,q+.192\,(-.047-.025536\,q)\\q&=1.3117.\end{aligned}$$

Whence

$$p=-.08049$$

These values give in (1bis)

$$\begin{aligned}r_{12}&=1.312-(.080\times.133\times.192)\\&=+1.310\end{aligned}$$

Assuming r_{12}, r_{15}, r_{25}, and r_{35} known and solving for r_{13} we get

$$\begin{aligned}q&=-.119\\p&=+.911\end{aligned}$$

These values in (1) give

$$r_{13}=+.908$$

As a correlation coefficient can never be greater than 1, $r=1.310$ is impossible. The computed values of r_{12} and of r_{13} are in both cases more than twelve times the observed values and opposite to them in sign. Such results are ridiculous.

Let us now test this system for the coefficient of determination of the causes which we considered as "all other causes," group 7. From the principle that the sum of the coefficients of determination must be unity, we have

$$(5)\ q^2+p^2+2\,qpll'+f^2=1$$

Substituting in this the values obtained for p and q in our first solution, we have

$$1.7213 + .0064 - .0054 + f^2 = 1$$
$$f^2 = -.7223$$

Substituting the values from the second solution, we have

$$f^2 = .1615$$

What does this really tell us about the effects of the factors not included? In the first case the determination is negative and has no meaning. The proportion of the standard deviation of the seed weight due to all other causes than those acting through seeds per pod and ovules per pod is $\sqrt{-.7223} = f$ according to WRIGHT'S theory. This is an imaginary standard deviation, a thing not encountered in statistics. In the second case we find that the unknown causes play apparently a real although small part in determining the seed weight. But there is no inherent or logical reason *in* WRIGHT'S *theory* why the first solution is not as good as the second.

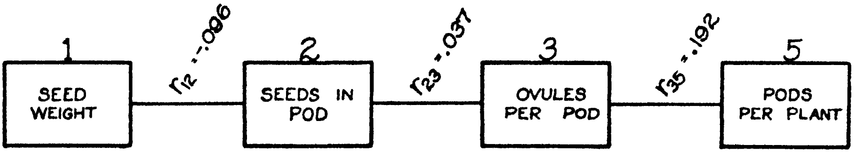

FIGURE 8.—An alternative set-up for the seed-weight example, giving observed correlation coefficients.

WRIGHT gives a special formula for finding the coefficient of determination of factors not specifically included in a system when correlations between the factors included are known. In our case the appropriate formula seems to be the one for two known correlated causes acting upon the effect. Using this formula and the observed r's gives the coefficient of determination between not-included causes and the effect, or f^2, equal to 0.9944, but using the calculated r's gives f equal $\sqrt{-1.482}$. Here is another case of two widely different values for the same constant, and one value is again imaginary and impossible.

It may be contended that the causal connections are really a straight line from pods per plant to ovules per pod, to seeds per pod, to seed weight. In this case we draw our diagram as in figure 8. If we multiply together the path coefficients from 1 to 5, we should obtain the correlation between 1 and 5 because there are no common causes and the path coefficients therefore are equal, on WRIGHT'S theory, to the coefficients of the correlation. By multiplying we find $r_{15} = -.000682$. This is not even one percent of the observed value. Evidently the theory works with this set-up no better than with the last.

Example 2

This is an application of the method of path coefficients in an attempt to determine approximately the relative importance of some factors influencing the amount of heat produced in human basal metabolism. As shown in figure 9, we assume that stature determines in part body weight and body surface, and that these, with a group of other factors, determine the heat produced. The correlation coefficients given in the figure are taken directly from Harris and Benedict (1919) with the exception of the one between surface and stature which had to be computed from the raw data given in the reference. This figure is identical in form with figure 7 used in example 1, and the same set of equations is therefore applicable. Without repeating these equations we will give the results obtained by solving them. Treating r_{12} as unknown we find its value to be +.3718, or less than one-half of the observed value. Treating r_{13} as unknown we find its value to be +.5997 or about three-fourths of the observed value. The correlation between the heat produced, and factors other than body weight and surface and those acting through them, is found to be either (a) $r_{10} = +.5703$, or (b) $\sqrt{-1.3893}$, or (c) +.338, depending upon the

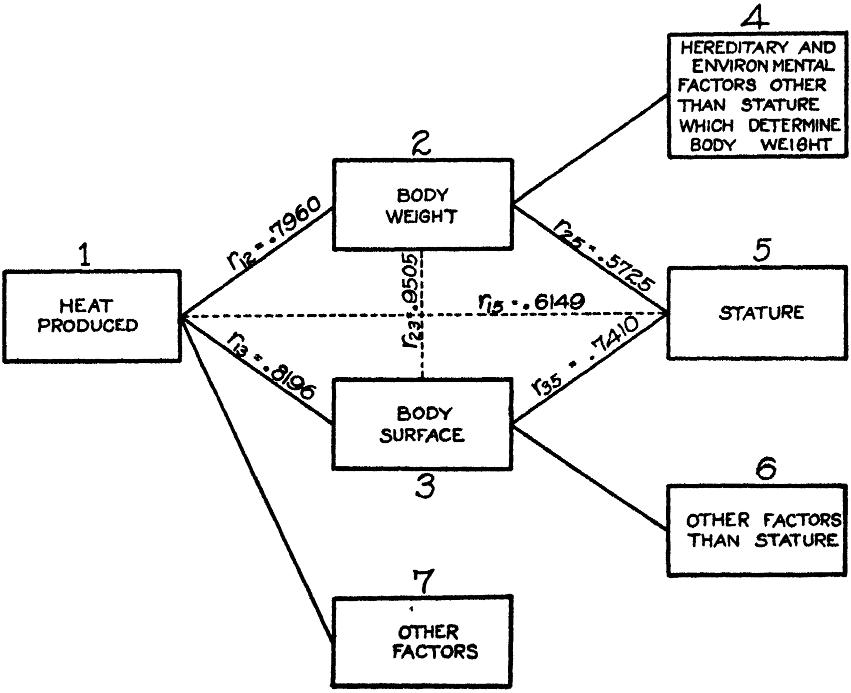

Figure 9.—The system set up for example 2, showing the observed correlation coefficients.

values used for r_{12}, and r_{13}. The values of the path coefficients are found to be $p=+.8072$ or $+.3196$, and $q=+.0293$ or $+.6604$. The results of this example, like those of the preceding, are inconsistent with themselves and with reality. When we see that the path coefficients are unreliable in these cases where we can check them, we are not likely to place any great confidence in them where they cannot be checked.

Should the criticism be made that these examples give absurd results because the true action of the variables upon each other is not truly represented in the diagram the writer would reply, first, that such criticism but strengthens one of his main points; namely, that it is impossible to tell *a priori* how the system should be set up, and that the closeness of agreement between calculated or expected and observed values is an unscientific criterion by which to judge the validity of such a system; and second he would invite careful examination from a biological standpoint of his diagrams and Wright's, with a view of the reader's seeing for himself whether the one set is more unfair or less related to the probable truth than the other.

CONCLUSION

We therefore conclude that philosophically the basis of the method of path coefficients is faulty, while practically the results of applying it where it can be checked prove it to be wholly unreliable.

The writer believes himself still open-minded on Wright's proposition, but has an even more intense conviction that before that author's contribution to the theory of partial correlation can be taken seriously he will have to bring forward evidence altogether more cogent in respect of both logic and fact than any he has so far adduced.

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THE CORRELATION BETWEEN FIRST- AND SECOND-YEAR EGG PRODUCTION IN THE DOMESTIC FOWL

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INTRODUCTION

It seems rather remarkable that the problem of the relationship between the egg production of the first and second year in the domestic fowl should have received no wider attention at the hands of practical poultrymen or experimental investigators. As far as we are aware the only quantitative discussions of the subject have been those of PEARL and SURFACE (1909), of NIXON (1912), and of BALL, ALDER and EGBERT (1916).

From the theoretical side the interrelationships between the physiological activities of the different periods of the life of the organism is a problem of independent importance. It is, furthermore, impossible to solve the theoretical problem of the mode of inheritance of fecundity in the fowl without detailed knowledge of the distribution of fecundity and of the interrelationships between the fecundity of diverse periods.

From the practical side it will be clear at once that if there be a marked correlation between the record of the first- and the second-year's production of the individual it will be possible to extend the method of prediction of future egg production, now being developed for the first year (HARRIS, KIRKPATRICK and BLAKESLEE 1921; HARRIS, KIRKPATRICK, BLAKESLEE, WARNER and CARD 1921), to second-year production. Such methods of prediction will be practicable[1] in the case of trap-nested birds or of birds selected by the color criteria first investigated in a detailed statistical manner by one of us in cooperation with BLAKESLEE, WARNER and KIRKPATRICK (HARRIS, BLAKESLEE and WARNER 1917; HARRIS, BLAKESLEE, WARNER and KIRKPATRICK 1917; BLAKESLEE, HARRIS, WARNER and KIRKPATRICK 1917).

It is possible, therefore, that a knowledge of either of these criteria will make possible the extension of the method of culling, with a resulting material increase of the second-year production.

In relation to the problem of commercial egg production, it may be noted that the bird's second-year production may in commercial work be a factor not to be ignored. Those who have dealt with the problem of the inheritance of fertility from the experimental side have in general tacitly assumed that only first-year records require consideration. But this has not been the case invariably; BALL, TURPIN and ALDER (1914), BALL, ALDER and EGBERT (1916) and BALL and ALDER (1917) fully recognized the desirability of obtaining more than the first-year record of the bird as a measure of the egg-producing capacity of the organism. BALL, ALDER and EGBERT (1916, p. 4) write:

> ". . . It seemed necessary, before intelligent selection could be practised to discover:
>
> First, a reliable measure of the productivity of a hen.
>
> Second, the average productive life of a hen (that should be considered in selection). With these two points established it was thought that it would then be possible to plan an experiment to determine the value of selection in improving a strain of birds."

HISTORICAL

GOWELL as early as 1903 recognized the importance of the problem of inter-annual correlation when he wrote (GOWELL 1903, p. 74):

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[1] While they may be scientifically practicable they may not be economically feasible in all commercial plants.

> "With many poultrymen the idea is prevalent that if a hen lay but few eggs the first year, she is likely to do better the second year, than though she had laid well during the first.
>
> The data so far secured does not show that hens that yield 120 eggs, or less, the first year, yield satisfactorily the second year. Those that yielded in the vicinity of 100 the first year have yielded very lightly the second year."

As far as we are aware the first determination of the relationship between the production of the first and second year of the life of the bird is that based by PEARL and SURFACE (1909, pp. 63-66) upon the data for the 66 Barred Plymouth Rock birds recorded by GOWELL (1903, p. 75). They state the correlation to be

$$r = +0.032 \pm 0.083$$

and conclude:

> "While this conclusion is positive, yet it is extremely small and obviously insignificant in comparison with its probable error. We can only conclude that so far as any evidence from our present material is concerned there is no sensible correlation between the egg production of the first laying year and that of the second."

PEARL (1912, p. 169) says:

> ". . . the potential 'anatomical' fecundity is very much higher than the actually realized fecundity. This is true even if we suppose the bird to be allowed to live until it dies a natural death. *Experience shows that birds which make a high fecundity record in the first year of their life, generally speaking, never do so thereafter.*"[2]

While this conclusion is at variance with the statements made by GOWELL in recording the original data, and is in itself demonstrably incorrect,[3] it seems to have been widely accepted by geneticists and has probably had a serious deterrent influence on the investigation of the economically highly important problem of the interrelationship of first- and second-year egg production. It has also tended to focus attention upon first-year record only in the investigation of the problem of inheritance of fecundity in the fowl. These problems will be discussed in detail later.

NIXON in 1910 clearly recognized (NIXON 1912) the essential problems of inter-annual correlation in egg production when she stated that the purpose of her study of records of 88 White Leghorns was

> ". . . . to show, first, whether hens which produce well for any particular year are likely to give good egg production for a longer period; second, whether it is possible to judge from the egg record of any particular year what is likely to be the egg production of the same hens during succeeding years."

---

[2] Not italicized in original.

[3] The decimal point has been misplaced at some step in the calculation of the coefficient. The correct value is $r = .327 \pm .974$, a constant in excellent agreement with many of those given later in the present paper.

The 88 birds considered gave a low average production[4] in each of the three years. The averages were 92.4, 96.9, and 86.2 eggs in the three years. Using a class interval of 25 eggs she deduced the following correlations between the production of the different years:

First and second, $r=0.548\pm0.050$
First and third, $r=0.153\pm0.070$
Second and third, $r=0.397\pm0.061$

She also considers the relationship between the production of the three individual years and the production of the entire period. These results do not, however, concern us in this place.

The most extensive series of studies of the production of birds for a period of years is that carried out at the UTAH AGRICULTURAL EXPERIMENT STATION. In 1914 BALL, TURPIN and ALDER analyzed the records of a seven-year-old flock of White Leghorn hens and their progeny. In a later publication BALL, ALDER and EGBERT (1916) considered, among other problems, the relationship between the egg records of first and second, first and third, and second and third years. In this paper they bring together the literature on the egg-laying capacity of birds in years subsequent to the first. Finally BALL and ALDER (1917) treat the problem of the seasonal distribution of egg production.

The constants deduced from the Utah data are in full agreement with those presented later in this paper in that they indicate a positive correlation between the egg production of the individual in various years. There are, however, a number of points of theory which fall outside the scope of the data upon which this paper is based; in consequence it seems wisest to postpone a detailed discussion of these data until a comparative treatment of the various series of egg records now being analyzed is possible.

MATERIALS AND METHODS

The records analyzed represent the entire first- and second-year egg-laying performance of 443 White Leghorn birds submitted in the first VINELAND INTERNATIONAL EGG-LAYING AND BREEDING CONTEST. Originally 540 birds were entered but a number died during either the first or second year.

Details concerning the regulations governing the contest and the conditions under which the birds were maintained may be derived from previous publications (LEWIS 1917, 1918; LEWIS, HANNAS and WENE 1920).

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[4] The records were derived from six flocks. In many cases these were under experimental treatment, and fed different rations or in a different manner.

The methods of statistical analysis are those in common use in modern higher statistics. Details are given where necessary.

ANALYSIS OF DATA

Inter-annual correlation for total production in the flock as a whole

We will first of all consider the correlation between the total annual production of the first and of the second year of the bird. The constants are the following:

First year	*Second year*
Mean = 174 05 ± 1 29	Mean = 139.79 ± 1.19
σ = 40 10 ± 0 91	σ = 37.05 ± 0.84
C.V. = 23 04 ± 0.55	C.V. = 26.50 ± 0.64

$r =$ 5485 ± 0 0224

This coefficient shows a medium degree of correlation between the first- and second-year production of the birds.

TABLE 1

Mean second-year production of birds making various records in first year and mean first-year production of birds making various records in second year.

RECORD OF FIRST YEAR	SECOND YEAR		RECORD OF SECOND YEAR	FIRST YEAR	
	f	Mean record		f	Mean record
0– 14	1	0.00	0–14	4	123 75
15– 29	—	———	15– 29	—	———
30– 44	—	———	30– 44	1	112 00
45– 59	—	———	45– 59	3	154 67
60– 74	3	110 00	60– 74	6	119 50
75– 89	5	90 60	75– 89	23	125 26
90–104	6	108 83	90–104	35	146 97
105–119	24	109 00	105–119	54	159 59
120–134	28	113.46	120–134	70	169 60
135–149	51	123 04	135–149	65	170 78
150–164	59	129 80	150–164	66	181 86
165–179	73	140 36	165–179	53	201 04
180–194	62	148 56	180–194	38	203 95
195–209	46	154 39	195–209	17	206 76
210–224	42	158 64	210–224	5	230 22
225–239	23	173 83	225–239	2	208 00
240–254	10	175 40	240–254	—	———
255–269	8	188 50	255–269	—	———
270–284	—	———	270–284	1	209 00
285–299	2	141 50	285–299	—	———

It is idle to attempt a discussion of the distribution of the mean egg production of the bird's second year associated with given records of the bird's first year without grouping the highly variable annual egg productions of the first year in classes. Adopting a class range of 15 eggs we have the frequencies and means of arrays shown in table 1.

Using grouped records for the first year and ungrouped records for the second year, we find the following correlation coefficient and regression equation.

$r_{E_1E_2} = 0.5387 \pm 0.0227, \quad E_2 = 53.046 + 0.499\, E_1$

For the grouped values of the second year and the ungrouped values of first year production, the numerical values are

$r_{E_1E_2} = 0.5541 \pm 0.0223, \quad E_1 = 91.104 + 0.593\, E_2$

The correlations calculated from the grouped frequencies are practically identical with those calculated from the ungrouped values. The constants have been recomputed with one of the two variables grouped, in order to have proper constants based on the same grouping as that employed in the regression diagrams.

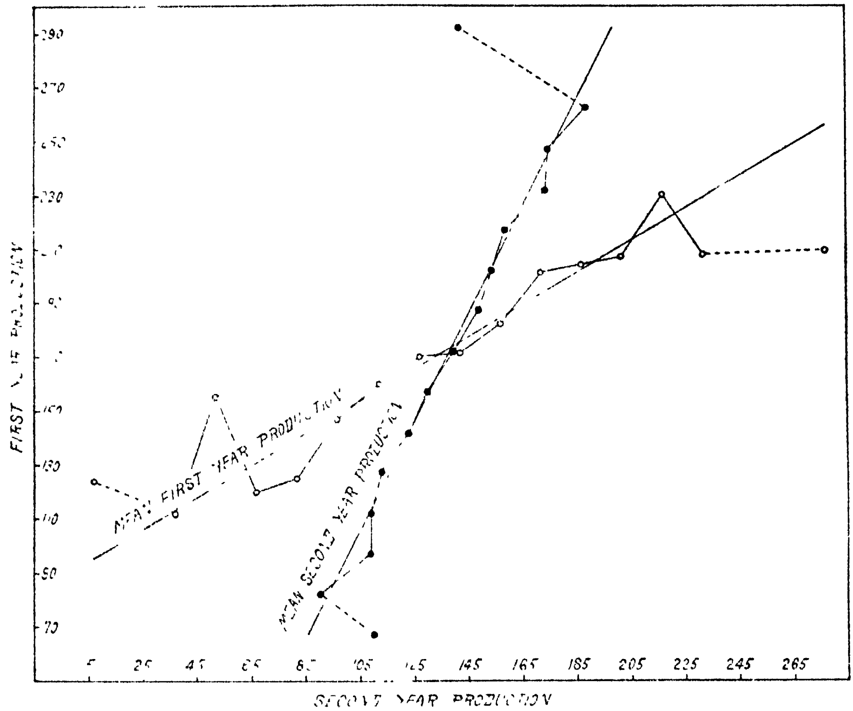

Diagram 1.—Straight-line regression equations showing relationship between first- and second-year production.

The two equations are represented in diagram 1 which shows the mean egg production for the first year associated with various records in the second year and the mean production of the second year associated with various records during the first year. The means are taken from table 1. Except for the higher and lower grades of production, the straight lines give excellent representations of the series of means.

For a formal test of linearity of regression we turn to the correlation ratio and BLAKEMAN's test for linearity of regression.

The values of η (PEARSON 1905) calculated from the formula

$$\eta = \sigma_{\bar{E}}/\sigma_E$$

where $\sigma_{\bar{E}}$ is the standard deviation of the mean egg production of the arrays and σ_E is the standard deviation of the egg production of the flock as a whole, serves as a measure of the interrelationship of the two variables whether regression be linear or not. BLAKEMAN's criterion (BLAKEMAN 1905)

$$\frac{\zeta}{E} = \frac{\sqrt{N}}{.67449} \cdot \tfrac{1}{2}\sqrt{\zeta} \cdot \frac{1}{\sqrt{1+(1-\eta^2)-(1-r^2)^2}},$$

when $\zeta = \eta^2 - r^2$, furnishes, in connection with Miss GIBSON's (1906) table, a convenient test for the linearity of the regression of the production of one year on the production of another year. The values of η and of BLAKEMAN's criterion are as follows:

For the regression of second-year production (ungrouped) on first-year production (grouped),

$$\eta = 0.5593 \pm .0220, \quad \zeta/E_\zeta = 2.39$$

For the regression of the first-year production (ungrouped) on the second-year production (grouped),

$$\eta = 0.5815 \pm .0212, \quad \zeta/E_\zeta = 2.81$$

In both cases ζ/E_ζ is not far from 2.5 and we may reasonably assume that regression is, within the limits of the errors of random sampling, practically linear in both cases.

Inter-annual correlation within the individual pens of the contest flock

Two suggestions may be offered in explanation of the correlation between the egg record of the first and second year of the individual birds, demonstrated in the foregoing section. First, that the correlation between the first- and second-year record of the bird is due to her own individuality with respect to egg-laying capacity within the flock from which she is drawn. Second, that the several flocks from which the exhibit birds are selected differ widely in both first- and second-year egg record, and that as a consequence of the combination of the pens from the various flocks there is a correlation between the first- and second-year production of the individual birds in the contest flock as a whole.

To determine to what extent this correlation is due to the individuality of the birds and to what extent it is attributable to the differentiation of the flocks from which the birds were drawn we may (a) employ the method of fractional inter-class correlation coefficients developed elsewhere (Harris 1913), and (b) determine the inter-annual correlation coefficients for the birds of each individual pen.

Table 2

Mean annual production for two years, and correlation between annual production for two years, in individual pens.

PEN	BIRDS IN PEN	MEAN FOR FIRST YEAR	MEAN FOR SECOND YEAR	$r_{E_1E_2}$	PEN	BIRDS IN PEN	MEAN FOR FIRST YEAR	MEAN FOR SECOND YEAR	$r_{E_1E_2}$
41	10	192.3	143.4	+.7004	68	9	166.3	139.2	+.5097
42	7	205.7	172.4	+.8842	69	8	190.8	133.3	+.6899
43	9	200.7	157.1	+.6356	70	9	179.7	152.7	+.0949
44	10	141.7	111.9	+.7596	71	10	182.0	143.4	+.3610
45	8	177.0	142.5	+.9208	72	7	172.1	160.6	+.5911
46	6	190.3	154.2	+.7538	73	5	104.6	155.6	−.0552
47	9	166.2	134.0	+.8316	74	10	164.6	123.2	+.8699
48	7	166.6	149.6	+.6733	75	9	151.8	127.0	+.8807
49	8	169.4	134.9	+.7168	76	9	212.2	173.3	+.2123
50	8	175.6	150.6	+.9182	77	8	170.0	140.9	+.9348
51	8	174.1	105.3	+.4575	78	7	169.6	148.0	+.8953
52	8	161.6	123.1	+.0398	79	9	159.4	127.0	+.7047
53	8	167.5	141.8	+.4117	80	10	163.6	144.0	+.8568
54	5	185.4	149.4	−.2563	81	10	186.2	135.9	+.8295
55	8	182.4	129.1	+.5755	82	8	184.5	145.6	+.6490
56	8	131.1	118.0	+.4974	83	9	173.0	131.3	+.5403
57	9	165.6	134.8	−.1037	84	7	147.7	109.0	+.6581
58	9	158.6	117.7	+.6773	85	10	130.5	124.8	+.7862
59	5	148.4	127.0	+.4880	86	10	167.8	146.6	+.5183
60	7	194.6	118.7	+.4526	87	8	152.9	109.6	+.6495
61	9	140.7	146.1	+.8362	88	9	225.0	143.9	+.7467
62	9	210.9	148.2	+.2480	89	6	199.2	111.0	−.4805
63	7	144.6	122.1	+.8095	90	7	201.3	140.4	−.0090
64	8	182.4	157.9	+.7217	91	6	159.8	119.3	+.6699
65	9	185.1	172.2	+.7186	92	8	202.6	180.5	+.2398
66	7	187.1	148.9	+.5066	93	10	187.8	154.5	+.2037
67	9	178.8	166.0	−.1529	94	10	188.5	145.6	+.8379

Using formulae xvi–xviii of the paper cited, letting x = first-year and y = second-year productions, and remembering that the number of birds is the same in each of the two years, we find for the correlation between the first-year record of a bird and the second-year record of the $n-1$ other individual birds (other than herself) of the same pen

$$r = +.0786 \pm .0318, \quad r/E_r = 2.5$$

The coefficient is positive in sign, and may be statistically significant. If it can be looked upon as statistically trustworthy it indicates that in the long run the first-year record of an individual bird furnishes some basis of prediction concerning the second-year record of other birds of the same pen. It is, however, very small as compared with the correlation, $r = .548$, for the two years of the individual bird's life.

This result proves that *it is the individuality of the bird within her own flock and not the individuality of the flocks which is the primary factor in determining the inter-annual correlation.*

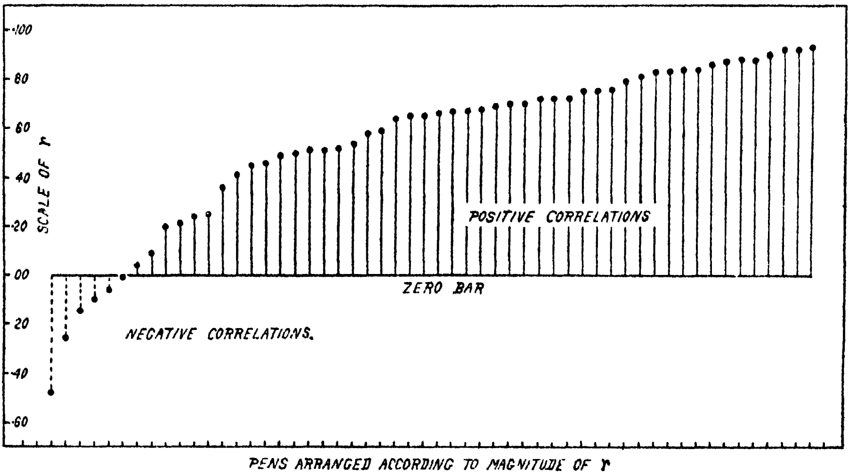

Diagram 2.—Distribution of magnitudes of correlation coefficients showing the relationship between the first- and second-year record of birds of the same pen.

The problem may be approached from the other side by the determination of the inter-annual correlation within the individual pens.

While correlation coefficients based on series in which ten is the maximum number, can have little value individually, it has seemed desirable to determine the correlation between the first- and second-year production of the birds of the individual pens of the Vineland Contest.

The correlations between the first- and second-year production within the individual flocks are shown in table 2. The first- and second-year means in this table will be used for another purpose later, and need not concern the reader for the moment. The coefficients are for the most part positive, and many of them are rather large. The distribution of the 54 coefficients, arranged in the order of their magnitude, are shown in diagram 2. A glance at the graph will show that only six pens show a negative correlation, and that the magnitude of these coefficients is small as compared with the mass of the positive coefficients.[5]

The mean value of the correlation between the first- and second-year production of birds of the same pen is

$$r = .5890 \pm .0307,$$

which is very nearly the same as the $r = .5485$ demonstrated for the whole 443 birds available for the two years.

Inter-annual correlation of flock means

From the practical standpoint it is important to determine whether a flock which gives a high yield in one year may be expected to be characterized by high production in the second year.

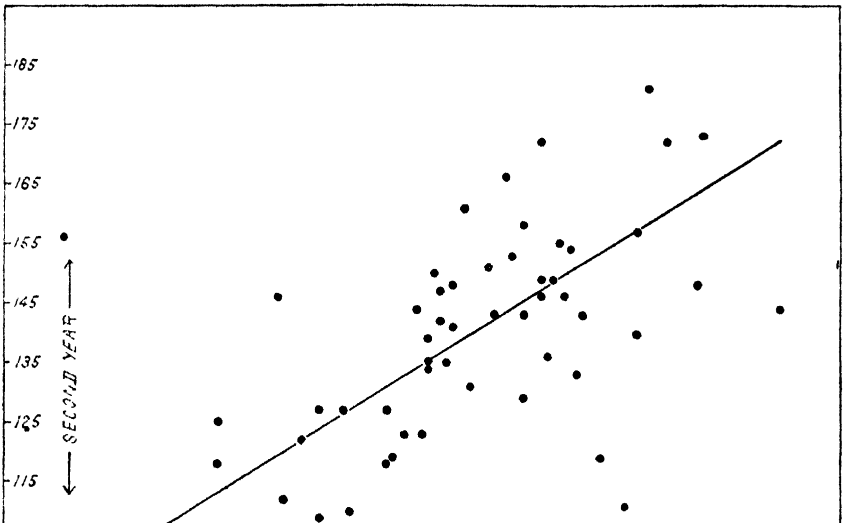

DIAGRAM 3.—Relationship between the average first- and second-year production of the pens.

Samples of ten birds or fewer are of course quite inadequate to represent the flocks from which they are drawn, and we must in consequence expect the correlation to be lower numerically than they would be found to be if based on means derived from larger numbers of birds.

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[5] The average number of birds per pen for the 48 pens showing positive correlation is 8.4 whereas the average number for the 6 pens showing negative correlation is 6.8. The smaller number of birds in the pens showing negative correlation, as well as the small magnitude of these correlations, suggests that the negative sign of these coefficients is due merely to the probable errors of random sampling when very small samples are available, rather than that it represents a true biological relationship.

Table 2 contains the pen averages for the two years and the number of individuals on which they are based.

The correlation between the average number of eggs laid in the first year, $\bar{E}_1$, and in the second year, $\bar{E}_2$, and the regression of the average number of eggs laid in the second year on the average number laid in the first year is

$$r_{\bar{E}_1\bar{E}_2} = 0.447 \pm 0.073,\quad \bar{E}_2 = 31.147 + 0.625\,\bar{E}_1$$

The means for the two years and the regression line are represented in diagram 3.

There is, therefore, a material relationship between the average production of the flock in the second year and the average production in the first year.

The correlation between the monthly records of one year and the annual record of another year

Because of the desirability of using the records of single months as bases for the prediction of the second year we have determined the correlations between the records made by the birds in the several months of the pullet year and the record made in the whole of the second year. For the sake of completeness, and to facilitate the investigation of certain problems to

TABLE 3

Comparison of inter-annual correlations for various months of first and second year.

MONTH OF FIRST OR OF SECOND YEAR	AVERAGE OF MONTHLY CORRELATION WHEN BIRDS ARE ARRANGED BY MONTHS OF FIRST YEAR	AVERAGE OF MONTHLY CORRELATION WHEN BIRDS ARE ARRANGED BY MONTHS OF SECOND YEAR	CORRELATION BETWEEN MONTHLY EGG RECORD OF FIRST YEAR AND ANNUAL EGG RECORD OF SECOND YEAR	CORRELATION BETWEEN MONTHLY EGG RECORD OF SECOND YEAR AND ANNUAL EGG RECORD OF FIRST YEAR
November......	.0967	.1063	+.1671 ± .0312	+.2162 ± .0306
December......	.1521	.0581	+.2802 ± .0295	+.1224 ± .0316
January........	.1645	.1674	+.3103 ± .0290	+.3234 ± .0287
February.......	.1448	.1689	+.2602 ± .0299	+.3228 ± .0287
March.........	.1302	.1944	+.2352 ± .0303	+.3240 ± .0287
April..........	.1181	.1025	+.2105 ± .0306	+.1497 ± .0313
May...........	.1527	.1342	+.2862 ± .0294	+.2081 ± .0307
June...........	.1779	.1493	+.3429 ± .0283	+.2436 ± .0301
July...........	.1943	.1826	+.3727 ± .0276	+.3272 ± .0286
August.........	.2222	.2020	+.4350 ± .0260	+.3717 ± .0276
September......	.1620	.2570	+.3390 ± .0284	+.4974 ± .0241
October........	.1798	.1727	+.3565 ± .0280	+.3469 ± .0282

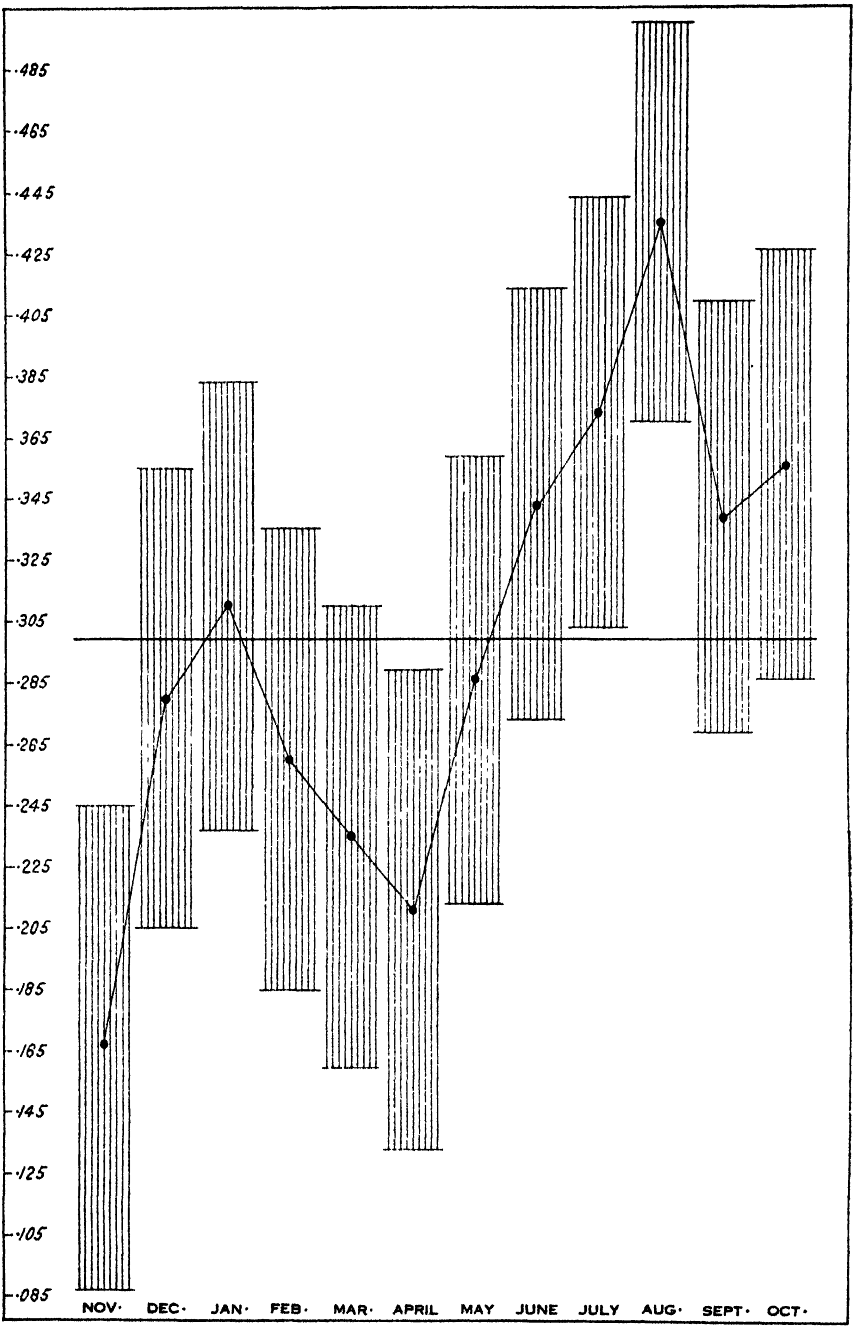


Diagram 4.—Magnitudes of correlation between the production of the individual months of the first year and the annual production of the second year.

be discussed later, we have also determined the correlations between the individual months of the second year and the annual production of the first year.

The coefficients are laid beside the means of the correlations between the individual months of the year (to be discussed later) in table 3.

The results for the correlation between the individual months of the first year and the annual total of the second year are represented graphically in diagram 4.

In this diagram the magnitude of the correlations for the individual months of the first year and the annual total of the second year is shown by the relation of the solid dots to the scale of ordinates. On either side of these dots a shaded area measures a distance of two and one-half times the probable error of r as determined from the formula

$$E_r = .6745\ (1-r^2)\ /\ \sqrt{443}.$$

The heavy transverse bar represents the average value of the twelve coefficients.

The correlation for the winter months is low, increasing from November to January, after which it drops to the spring minimum in April, and then rises to the maximum values for the year in the summer months of July and August.

It appears from this diagram that the magnitude of the correlations between the individual months and the total productions of another year of the bird's life differs significantly from month to month. This conclusion is based in part on a study of the probable errors and in part on the general consistency of the results, taken alone and in comparison with those of a following section. A similar diagram has been prepared for the relationship between the individual months of the second year and the total production of the first year, but is so similar to diagram 4 that it does not merit reproduction.

The suggestion may be offered that the differences between the correlations for the records for individual months of one year and the annual totals of another year may be due to differences in the significance of the correlation coefficient. There might be a true biological relationship between the egg production of a given month of one year and the total production of another year, which would not be detected by the correlation coefficient at all if regression were non-linear. It seems desirable, therefore, to test the linearity of the regression of the annual production of one year on the production of the individual months of the other year, with the object of determining whether the differences in the magnitude of the correlations between the monthly records of one year and the annual records of another year may be due to inadequacies in the correlation coefficient as a measure of the interrelationship in the egg-laying activities of these two periods.

Since the regression of the second-year annual record on the first-year monthly record is the one of practical significance in the prediction of future production, it alone will be considered. The regression equations are as follows:

$$
\begin{aligned}
E &= 131.088 + .835\,e_1\\
E &= 126.040 + 1.333\,e_2\\
E &= 124.810 + 1.562\,e_3\\
E &= 120.150 + 1.648\,e_4\\
E &= 94.382 + 2.228\,e_5\\
E &= 89.272 + 2.320\,e_6\\
E &= 81.649 + 2.635\,e_7\\
E &= 83.325 + 2.639\,e_8\\
E &= 93.752 + 2.311\,e_9\\
E &= 104.718 + 2.236\,e_{10}\\
E &= 129.102 + 1.657\,e_{11}\\
E &= 131.734 + 1.958\,e_{12}
\end{aligned}
$$

These lines, and the mean productions of the birds for the second year for each grade of monthly egg record for the first year, are represented in diagrams 5 and 6. Certain of the means deviate very widely from the theoretical lines. These are, however, for the most part based on only a few birds, and in some cases upon only a single individual. Until further data are available, it does not seem worth while to consider regression curves of a higher order.

From the practical side it is very fortunate that the highest correlations between the production of the individual months of the first year and the annual total of the second year is found in the summer months of July and August. It is in the months of June, July and August that culling should be undertaken for the purpose of eliminating from the flock birds which will not be useful for further production during the current (pullet) year. It is at this time that the best selection of birds for the second-year's production can be made.

The practical phases of this problem of the prediction of second-year production from the records of individual months of the first year will be treated in an Experiment Station bulletin to be published shortly. The correlations between the monthly records of the first and second year will be discussed in the following section.

Inter-annual correlation for monthly production

We now have to consider the correlation between the production of the individual months of the bird's first and second year. Table 4 shows the

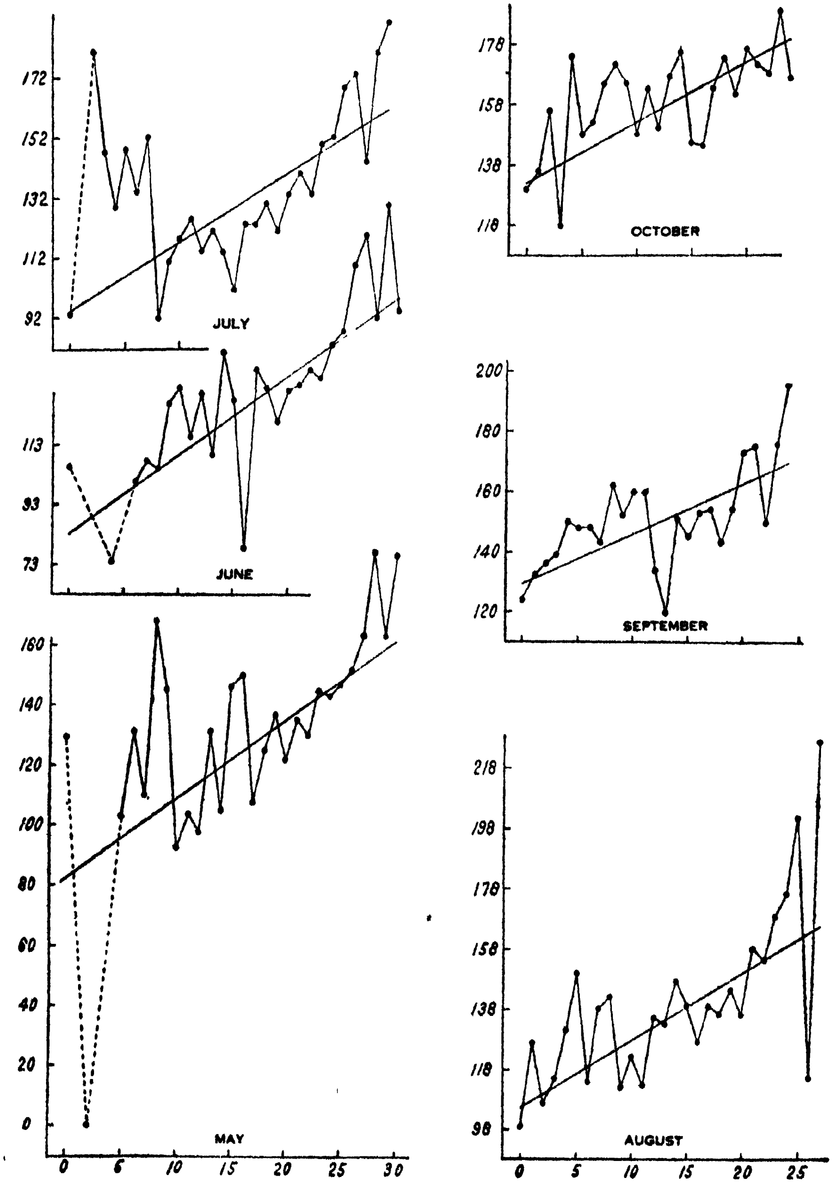


Diagram 5.—Regression of second-year annual production on first-year monthly production.

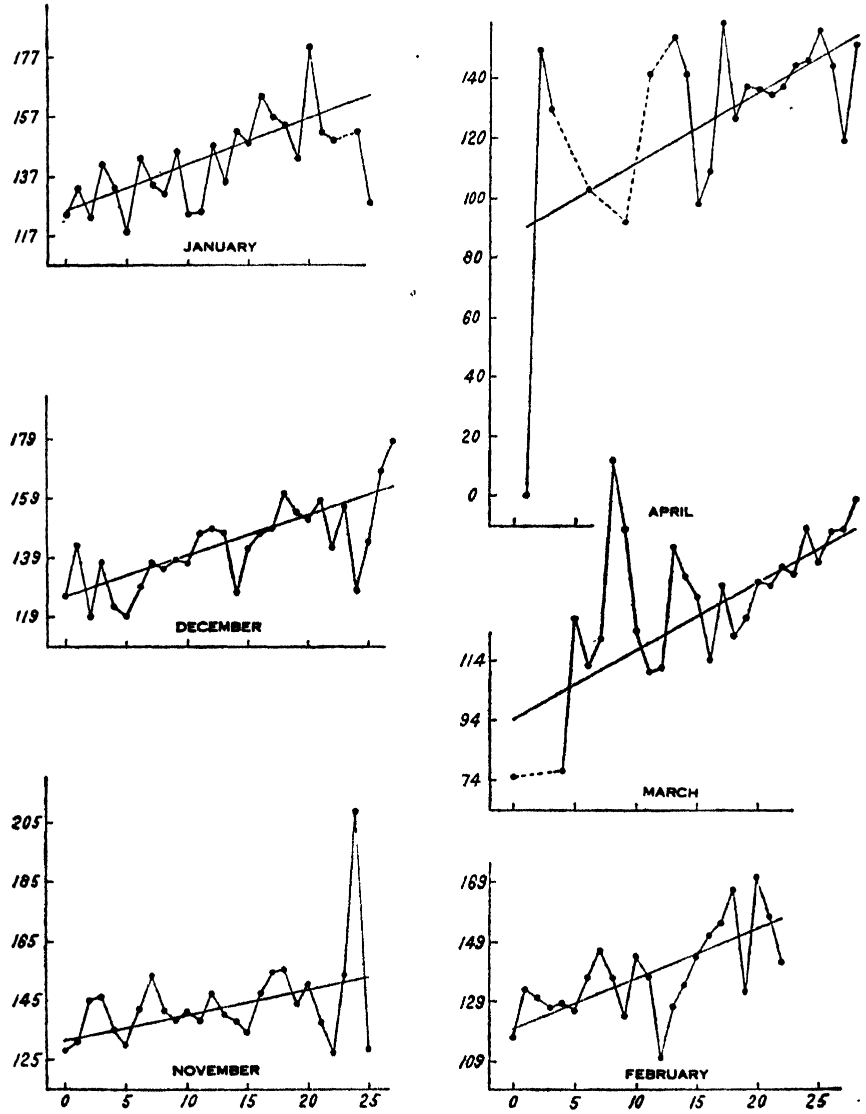


Diagram 6.—Regression of second-year annual production on first-year monthly production.

TABLE 4

Correlation coefficients measuring the relationship between the egg production of the individual months of first two years of the bird's life.

Bird's second year

Bird's first year	NOVEMBER e_1	DECEMBER e_2	JANUARY e_3	FEBRUARY e_4	MARCH e_5	APRIL e_6
November	+.1100± .0317	+.0746± 0319	+.1627± .0312	+.2053± 0307	+.1686± 0311	+.0410± .0320
e_1.......	3.47	2 34	5.22	6.69	5.41	1.28
December	+.2017± .0307	+.1685± .0311	+ 2195± .0305	+.2714± 0297	+.1504± .0313	+.0184± .0320
e_2........	6.56	5.41	7.19	9.14	4 80	0.57
January	+.2031± 0307	+.1713± .0311	+ 3186± .0288	+.2975± .0292	+ 1656± .0312	−.0423± .0320
e_3........	6 61	5 51	11.1	10.2	5 31	1.32
February	+.1605± .0312	+.2003± .0308	+.3316± .0285	+.3504± .0281	+ 2373± .0302	−.0348± .0320
e_4........	5.14	6.51	11.6	12.5	7.84	1.09
March	+.0744± .0319	+.0215± .0320	+.1509± .0313	+.2382± .0302	+.3348± .0285	+ 0938± .0318
e_5........	2.33	0 67	4.82	7.88	11.8	2.95
April	+.0325± .0320	−.0290± .0320	+.0476± .0320	+.1031± .0317	+.3175± .0288	+.2155± .0306
e_6........	1.02	0.91	1.49	3.25	11.0	7.05
May	+.0166± .0320	−.0265± .0320	+.0339± .0320	+.0280± .0320	+.1922± .0309	+.2810± .0295
e_7........	0 52	0.83	1 06	0.87	6.23	9.52
June	−.0177± .0320	+.0165± .0320	+.0949± .0318	+.0182± .0320	+.1842± 0310	+.2686± .0297
e_8........	0.55	0 52	2.99	0.57	5.95	9 03
July	−.0444± .0320	+ 0353± .0320	+.1961± .0308	+.0641± .0319	+.2147± 0306	+.2077± .0307
e_9........	1.39	1.10	6.36	2.01	7 02	6.77
August	+.0814± .0318	+.0893± .0318	+.2008± .0308	+.1182± .0316	+.1661± 0312	+.1407± .0314
e_{10}.......	2.56	2.81	6.53	3.74	5 33	4.48
September	+.0678± 0319	−.0439± .0320	+.1265± .0315	+.1314± .0315	+ 1069± .0317	+.0303± .0320
e_{11}.......	2.13	1.37	4.01	4.17	3 37	0.95
October	+.3895± .0272	+.0192± .0320	+.1254± 0315	+.2013± 0308	+ 0940± .0318	+ 0103± 0320
e_{12}	14 3	0 69	3 97	6 54	2 96	0 32

144 correlation coefficients which may be deduced as measures of this relationship.

For the purpose of determining how close the relationship between the months of the first two years of the bird's life may be, we have formed the frequency distribution of the magnitudes of the correlation coefficients shown in table 5.

These coefficients are preponderantly positive in sign. Two coefficients fall in the "zero" class, ten are negative in sign and numerically larger than −.0125, while 132 are positive in sign and larger than +.0125 numerically.

When r is very small the probable error is difficult of interpretation. We have, however, calculated the probable errors of the 144 individual coefficients by the usual formula. These probable errors are given in table 4. The ratios of the correlation coefficients to their probable errors are given (to one or two decimal places) under the individual coefficients. A glance at these entries in table 4 will show that many of the coefficients are over three times as large as their probable errors. These tests of significance (r/E_r) have been seriated according to their magnitude in table 6.

TABLE 4 (continued)

Correlation coefficients measuring the relationship between the egg production oj the individual months of first two years of the bird's life.

Bird's second year

Bird's first year	MAY e_7	JUNE e_8	JULY e_9	AUGUST e_{10}	SEPTEMBER e_{11}	OCTOBER e_{12}
November	+.0765± 0319	+.0532± 0320	+ 0220± 0320	+ 0278± 0320	+.1256± .0315	+.0932± .0318
e_1	2 40	1 66	0 69	0 87	3 98	2.93
December	+.0375± 0320	+ 0695± 0319	+.0889± 0318	+.1481± 0313	+ 2612± 0299	+.1902± .0309
e_2	1.17	2 18	2.79	4 73	8.75	6 16
January	−.0384± .0320	+ 0283± .0320	+ 1245± 0316	+ 2008± 0308	+ 3208± .0288	+ 2237± 0304
e_3	1.20	0 89	3 95	6 53	11.2	7 35
February	−.0651± .0319	+ 0249± 0320	+ 0989± 0317	+ 1202± 0316	+ 2124± 0306	+.1014± 0317
e_4	2 04	0 78	3 12	3 81	6 94	3 20
March	+.0789± 0319	+ 0895± 0318	+ 0974± 0317	+ 1401± .0314	+ 1610± .0312	+ 0815± .0318
e_5	2 48	2 82	3 07	4 46	5 16	2.56
April	+.1774± .0310	+ 1560± 0313	+.1062± 0317	+ 1108± 0317	+ 1164± 0316	+ 0630± .0319
e_6	5 71	4.99	3 35	3 50	3 68	1 97
May	+.3369± 0284	+ 3191± 0288	+ 2317± 0303	+ 1979± 0308	+ 1425± 0314	+.0796± .0318
e_7	11.9	11 1	7 64	6.43	4 54	2.50
June	+ 3929± 0271	+.3335± .0285	+ 3056± 0291	+ 2810± 0295	+.2019± .0307	+.0549± 0320
e_8	14 5	11 7	10 5	9 52	6.57	1.72
July	+.3642± 0278	+ 3869± 0273	+ 3665± 0277	+ 2554± 0300	+.2183± 0305	+ 0673± 0319
e_9	13 1	14 2	13.2	8.53	7.15	2 11
August	+.2669± 0298	+ 2772± 0296	+ 3652± 0278	+ 3410± 0283	+ 3654± 0278	+.2547± 0300
e_{10}	8 97	9 37	13 1	12 0	13 2	8 50
September	+.0160± .0320	+ 0482± 0320	+ 2557± 0300	+ 3354± 0284	+ 4791± 0247	+ 3905± 0272
e_{11}	0 50	1 51	8 54	11 8	19 4	14 4
October	− 0332± 0320	+ 0056± 0320	+ 1286± 0315	+ 2651± 0298	+ 4799± 0247	+ 4718± 0249
e_{12}	1 04	0 18	4 08	8 90	19 5	18 9

This table shows that over a hundred of the positive coefficients may be considered significant in comparison with their probable errors.

Turning now to the series of correlation coefficients as a whole we may note that the statistical constants deduced from the distribution of the correlation coefficients (in table 5) are:

Mean,	.1585 ± .0070
Standard deviation,	.1242 ± 0049
Coefficient of variation,	78.36

It is clear, therefore, that the average value of the correlation between the individual months of the bird's first and second year is positive and deviates significantly from zero. We may confidently assert, therefore, that the relationship between the egg production of the bird's two years is so close that, within limitations to be discussed presently, any month of the first-year record furnishes some (though very slender) basis for prediction of the egg production of each individual month of the bird's second year.

It will be noted that the standard deviation of the coefficients is relatively very large, 78.36 percent of the mean. If the correlation between all the months of the two years were biologically the same, and differences between the calculated values due merely to the probable errors of random sampling, one might expect a standard deviation of

$$\sigma'_r = \frac{1-r^2}{\sqrt{N}}$$

TABLE 5

Frequency distribution of correlations between the individual months of the bird's first and second year.

CLASS CENTERS	FREQUENCIES
−.075	1
−.050	4
−.025	5
.000	2
+.025	17
+.050	5
+.075	12
+.100	15
+.125	9
+.150	9
+.175	8
+.200	14
+.225	7
+.250	5
+.275	7
+.300	2
+.325	9
+.350	2
+.375	5
+.400	3
+.425	—
+.450	—
+.475	3

TABLE 6

Frequency distribution of ratios of correlation coefficients for individual months to their probable errors.

CLASS	FREQUENCIES
− 2.50 to 1.50	1
− 1.50 to .50	9
− .50 to .50	3
+ .50 to 1.50	18
+ 1.50 to 2.50	10
+ 2.50 to 3.50	19
+ 3.50 to 4.50	12
+ 4.50 to 5.50	12
+ 5.50 to 6.50	7
+ 6.50 to 7.50	15
+ 7.50 to 8.50	4
+ 8.50 to 9.50	8
+ 9.50 to 10.50	3
+10.50 to 11.50	5
+11.50 to 12.50	7
+12.50 to 13.50	4
+13.50 to 14.50	4
+14.50 to 15.50	—
+15.50 to 16.50	—
+16.50 to 17.50	—
+17.50 to 18.50	—
+18.50 to 19.50	3

where σ'_r is the theoretical standard deviation of r as determined on samples of $N = 443$ individuals. Taking the mean value of the correlation in the 144 permutations of months as the most probable value of r, this formula gives

$$\sigma'_r = .0463,$$

as compared with $\sigma_r = .1242$ determined directly from the constants.

It is evident that there is *differentiation* in these correlation coefficients, not merely *variations* due to the errors of random sampling. This problem will be treated in detail in a forthcoming paper now in preparation. One aspect of the problem will be discussed in the following section.

Genetic significance of the inter-annual correlation for monthly production

We now have to consider these correlations in relation to a genetic problem, namely, that of the dependence of fertility on the presence or absence of definite Mendelian factors.

There has become current among geneticists the theory of a "winter cycle" of egg production which is often assumed to have become superimposed upon the normal "spring" or "reproductive cycle." Statements concerning the exact relations of this "cycle" to the other "cycles" of the year have left much to be desired in definiteness. A detailed review of the theory of the "winter cycle" in relation to the problem of total egg production and to the inheritance of egg production would lead us too far from our main purpose. It should be clear, however, that if birds differ in their egg production because of the presence or absence of definite Mendelian factors for winter egg production, there should be a correlation between the winter egg production of the bird's first year and that of the bird's second year. This should be true unless an additional hypothesis be adduced, namely that the genes which determine differences in the winter egg production of the pullet year have no influence on the egg production of the second year. If superiority or inferiority in laying capacity be determined primarily by these Mendelian factors for winter production, it would be reasonable to expect that the highest correlations between the production of the sub-periods of two years would be found in those periods which owed their differences to actual genotypic constitution, providing always that the second hypothesis suggested above is not adduced to explain away a result contrary to this reasonable expectation.

In short, the assumption that variation in winter egg production is largely due to zygotic composition with reference to three distinct factors which are separately inherited according to Mendelian theory seems to have certain consequences for inter-annual correlation. The egg record of the second year is distributed over a winter, spring, summer and autumn period although we have not at present evidences to indicate that the egg records of these arbitrarily delimited periods represent real biological entities. If the winter egg production of a bird's first year is determined by her zygotic formula it seems rather difficult to assume that her second period of winter production is independent of this zygotic formula. If the egg record of the winter period or cycle in both first and second year be determined by zygotic constitution, which according to assumption may differ fundamentally from individual to individual, *one might logically expect to find an abnormally high correlation between the winter months of the entire winter periods of the two years even though the correlations for all the other months were sensibly zero.*

Our first problem is, therefore, to determine whether, as a matter of fact, there is a higher correlation between the months comprising the winter "cycles" of the first and second year than between the months of the spring, summer or autumn "cycles."

To solve this problem we have first of all determined the mean correlations between the months of the first and second year, for each month of the first year and each month of the second year, respectively. The results are shown in the first two sections of table 3. These are the averages of the twelve months of the second year associated with November, December, etc., of the first year, and of the correlations between November, December, etc., of the second year and the twelve individual months of the first year.

Our problem is to determine whether any group of these correlations can be looked upon as sensibly larger than any other group. It is evident from the tables that the averages differ somewhat among themselves. This would be expected because of the errors of random sampling, whether there be any real biological differentiation in the correlations in the individual months or not. We have, therefore, determined the standard deviation of the coefficient of correlation between the individual months of the two years from the frequency distribution shown in table 5. We have assumed for purposes of graphic representation that if the correlations for the various permutations of the months of the first and the second year were the same within the limits of the probable errors of random sampling, samples of twelve should show means with probable errors of $.6745\ \sigma_r/\sqrt{12}$.

On diagram 7 the heavy transverse lines represent the average correlation ($\bar{r} = .1585$) for the 144 combinations of first- and second-year months. The solid dots connected by lines show the values of the individual averages. The lines limiting the shaded area, above and below, show the limits of 2.5 times the probable error of the mean of samples of twelve. The light lines traversing the shaded zones are drawn to represent units of one-half the probable error.

From diagram 7 it appears that in each arrangement of the data only two averages fall outside the range of two and one-half times the probable error of the mean. In the determination of the averages according to the months of the first year there are seven determinations which fall within the range of the probable error above and below the general mean as compared with six cases within and six cases beyond this limit which would be expected if the correlation for the individual months were random

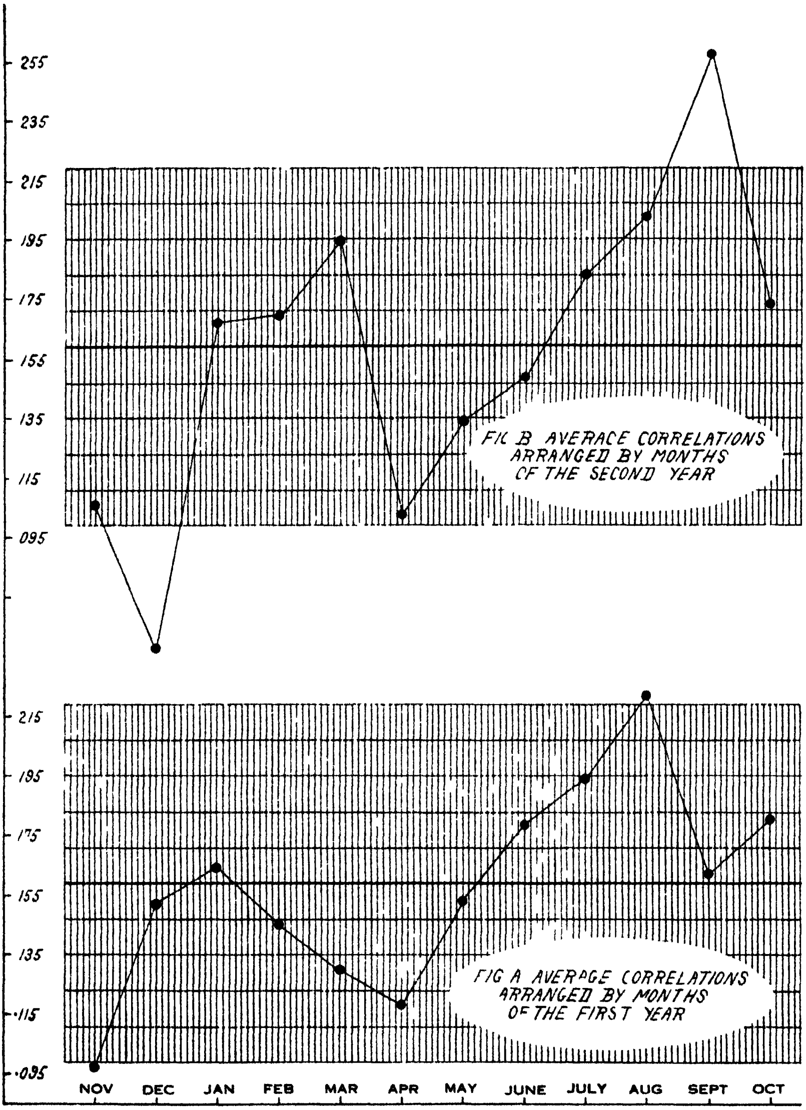


Diagram 7.—Average values of inter-annual correlations for production of individual months.

samples from a series of correlations with the variability of the present series. We can not, therefore, positively assert on the basis of the probable errors available that there is any significant difference between the average values of the inter-annual correlations of the individual months of either the first or the second year.

There is, nevertheless, at least an apparent orderly trend in the averages. In both series, November and April are conspicuously low, and the values of the averages rise from April to August or September. The average values of the inter-annual coefficients for November, December, January and February, *which, according to the Mendelian theory reviewed, should be higher than those for the other months, are as a matter of fact among the lower values*, all four coefficients for each of the two arrangements falling either below or very near the general average value of the 144 coefficients representing all the 12×12 permutations for the individual months of the first and second years.

As a further test of the differences in the correlations between the individual months, we may turn back to (a) the coefficients measuring the relationship between the production of individual months of the bird's first year and the total production of the bird's second year, and (b) the correlation between the individual months of the bird's second year and the total production of the bird's first year, as shown in the two final columns of table 3 and represented graphically in diagram 4 for the individual months of the first year.

The correlation coefficients measuring the relationship between the egg production of the individual months of the first year and the annual total of the second year show a distribution in close agreement with that found in figure A of diagram 7.

The results for the relationship between the individual months of the second year and the total production of the first year, shown in the final section of table 3 (but not represented graphically), are in excellent general agreement with the distribution of means of inter-annual correlations for individual months shown in figure B of diagram 7.

The preceding methods have the disadvantage that the entire range of months of the second-year's production associated with each month of the first year is lumped together. It is quite conceivable that the 12 general averages of correlations thus obtained do not properly represent the relation between the individual "cycles" of the first year and the individual "cycles" of the second year. It is quite conceivable that the average correlation for twelve months might give a quite erroneous measure of the correlations for the individual months. Nevertheless some average values must be considered because of the large probable errors of the constants when such fine divisions of the year are recognized.

It seems desirable, therefore, to determine the average value of the correlations between the months constituting the recognized "cycles" of production of the first year and the individual months of these "cycles" in the second year.

The periods of egg production, as classified by PEARL and SURFACE (1909) and adopted by other students of egg distribution are:

Period of winter egg production, November to February, inclusive.
Period of spring egg production, March, April and May.
Period of summer egg production, June, July and August.
Period of autumn egg production, September and October.

The averages for the correlations between the individual months of the different periods, and the standard deviations of these correlations, calculated directly from the ungrouped coefficients, appear in table 7. The values with signs are the averages of the correlations. The values without signs are the standard deviations.

TABLE 7

Average and standard deviations of inter-annual correlations for monthly periods classified according to conventional "cycles" of the first and second years.

		Second year			
		WINTER	SPRING	SUMMER	AUTUMN
First year	Winter (November to February)	+.21545 .07656 $N=16$	+.05954 .09583 $N=12$	+.08392 .05454 $N=12$	+.19105 .07516 $N=8$
	Spring (March to May)	+.05760 .07267 $N=12$	+.22534 .09324 $N=9$	+.16098 .07189 $N=9$	+.10732 .03559 $N=6$
	Summer (June to August)	+.07105 .07303 $N=12$	+.24512 .08180 $N=9$	+.32359 .04336 $N=9$	+.19376 .10733 $N=6$
	Autumn (September to October)	+.12713 .12184 $N=8$	+.03737 .04874 $N=6$	+.17312 .12061 $N=6$	+.45536 .03756 $N=4$

The reader who desires to do so may compare the averages for any two groups by calculating their difference in average correlation and the probable error of their difference by the usual method. Because of the relatively small number of correlations in each case we have preferred to test for differentiation between the several groups in a graphic manner.

We have worked on the assumption that, if the periods be undifferentiated, the probable error of the mean correlation for each period may be logically found from

$$E_{\bar{r}} = .6745\ \sigma_r/\sqrt{n}$$

where σ_r is the standard deviation of the 12 × 12 values of r and n is the number of permutations of months in the associated cycles of the two years. These numbers are shown beneath the means and standard deviations on table 7.

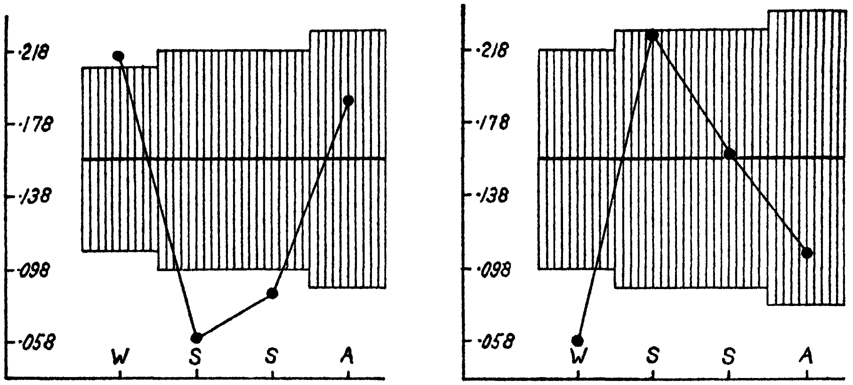
Remembering that for the 144 combinations of first- and second-year monthly records as deduced from the grouped correlation coefficients,

$$\bar{r} = .1585, \qquad \sigma_r = .1242$$

we have represented the mean value of the 144 constants by the position of the heavy bar on the four figures of diagram 8. On either side of the mean value we have shaded a zone limited by parallel lines above and below the general mean a distance of two and one-half times the probable error of the mean of samples of the number of correlations involved in the series of permutations in question.

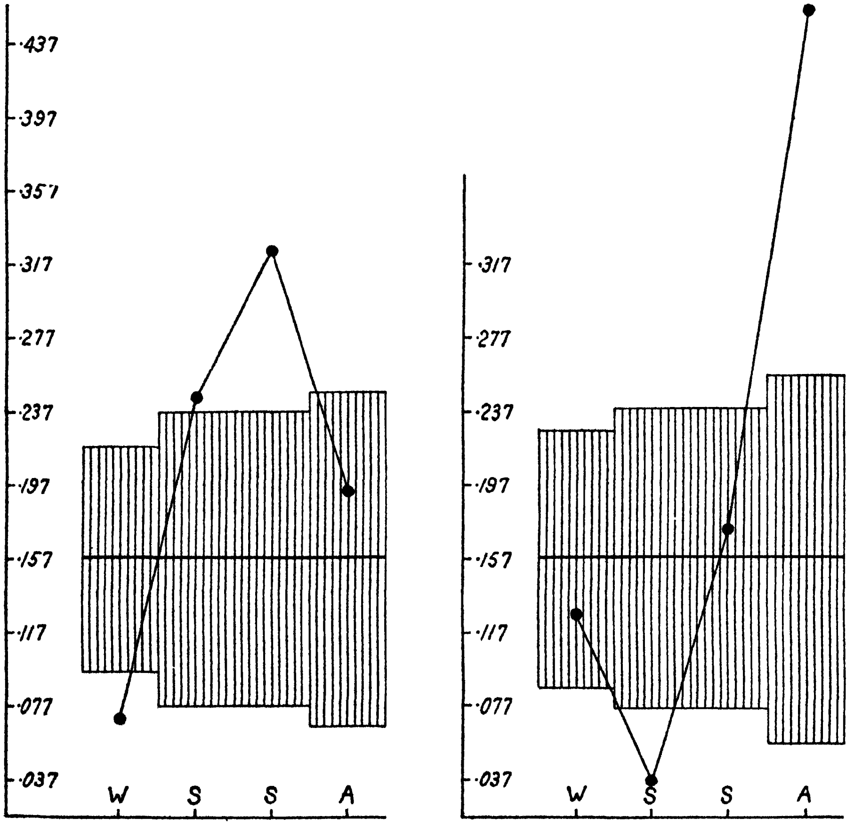
Inspection of the four figures of diagram 8 shows at once that there is a distinct individuality of the birds with respect to distribution of egg production throughout the year. The figure for winter production, for example, shows that there is a closer correlation between the winter production of the first year and both the winter and autumn production of the second year than between the winter production of the first year and either the spring or summer production of the second year. The diagram for spring production shows that there is a closer correlation between the spring production of the first year and the spring production of the second year than between the spring production of the first year and the record of any other of the three formal periods of the second year, although the spring and summer periods are more closely correlated than either spring and winter or spring and autumn.

Similar results are found for the average of the correlations between the summer months of the first year and the months of the various periods of the second year. The lowest average correlation is that between the summer months of the first year and the winter months of the second year. The correlations between the summer months and the autumn months average somewhat higher than those of the summer and winter months. Finally the correlations between the summer months of the first year and the spring and summer months of the second year are the highest of the four combinations.



WINTER PRODUCTION

SPRING PRODUCTION



SUMMER PRODUCTION

AUTUMN PRODUCTION

DIAGRAM 8.—Average values of inter-annual correlations for the "cycles" of the first and second year.

Comparable relationships are found when the averages of the correlations between the autumn egg records of the first year and those of the four groups of the second year are compared. The lowest average is that for autumn months and spring months. The average for autumn and summer months and for autumn and autumn months are the highest.

Summarizing the results shown by these diagrams, it is clear that (a) the average correlation for the months of the same period in the two years is in each case the highest average found in the series, that (b) the average of the correlations between the months of periods closely associated is in general higher than that of periods which are more widely separated in time, but that (c) an exception to this rule is found in the case of winter and autumn periods, which show a tendency to correlation with each other although they are the most widely separated periods. To a discussion of this point we shall return presently. For the moment it is sufficient to note that the averages show that there is a higher correlation for homologous periods of the two years. The results for the two figures of diagram 7 are therefore in some degree misleading. More detailed statistical analysis brings out relationships which are not obvious when the results are simply lumped according to the months of one year only.

Returning to the question of the significance of the deviations of these individual averages from the general average as indicated by their position with reference to the shaded area, we note that a number of the deviations would not, individually regarded, be considered statistically significant. The conclusions drawn above from the graphic tests for differentiation must rest primarily upon the consistency of results in the four diagrams. The important feature of this phase of the investigation is the conspicuous difference between the averages for the winter and spring "cycles" on the one hand and the summer and autumn "cycles" on the other. Notwithstanding the generally smaller numbers of correlations averaged in the two latter groups, the deviations from the general mean are much more conspicuous and more clearly significant. *The birds are, therefore, not primarily differentiated by winter production but by summer and autumn production.* So far as these evidences go, therefore, they suggest that in the White Leghorn heritable differences are to be looked for in the summer- and autumn-months' rather than in the winter-months' production.

We note that in this regard diagrams 7 and 8 are confirmatory. Both indicate a material increase in the closeness of correlation as the records are taken from successively later months.

The graphic method heretofore employed has the advantage that it brings out clearly the differentiation of the different periods with respect to the intensity of correlation between the two years. To test the significance of these differences we must have recourse to a formula due to PEARSON (1906). This formula has already been suggested as a test of prepotency in inheritance studies (HARRIS 1911).

PEARSON has shown that the difference between the average of a sub-sample and the average of the sample from which it is drawn is given by

$$(\bar{r}_p - \bar{r}) \pm .67449 \sqrt{\frac{\sigma_r^2}{N} + \frac{\sigma_{r_p}^2}{n}\left(1 - \frac{2n}{N}\right) - \frac{n(\bar{r} - \bar{r}_p)^2}{N(N-n)}}$$

where $\bar{r}_p$ is the mean correlation in the sub-class of n individuals from the population N showing the average correlation $\bar{r}$.

The results of this test are shown in table 8.

TABLE 8

Test of significance of deviation of the average correlation for the months of the conventional cycles from the general average for all months.

PERIODS	BIRDS' FIRST AND SECOND YEAR	
	Difference and probable error $\bar{r}_p - \bar{r} \pm E_{(\bar{r}_p - \bar{r})}$	Ratio of difference to probable error $\frac{\bar{r}_p - \bar{r}}{E_{(\bar{r}_p - \bar{r})}}$
Winter—Winter	+.0575±.0133	+ 4.327
Winter—Spring	− 0984±.0183	− 5.371
Winter—Summer	− 0740±.0119	− 6 240
Winter—Autumn	+ 0331±.0183	+ 1 813
Spring—Winter	−.1003±.0146	− 6.887
Spring—Spring	+ 0674±.0208	+ 3 243
Spring—Summer	+ 0030±.0166	+ 0 182
Spring—Autumn	− 0506±.0117	− 4.341
Summer—Winter	− 0869±.0147	− 5 931
Summer—Spring	+ 0872±.0185	+ 4 709
Summer—Summer	+ 1656±.0112	+14.775
Summer—Autumn	+ 0358±.0291	+ 1 229
Autumn—Winter	− 0308±.0283	− 1 091
Autumn—Spring	− 1206±.0145	− 8.292
Autumn—Summer	+ 0152±.0325	+ 0.466
Autumn—Autumn	+ 2974± 0139	+21 470

The entries in this table show that statistically significant deviations (> 2.5 times the probable error of the difference) of the subgroups of correlations above the average for all of the correlations, occur only in the winter-winter, spring-spring, summer-spring, summer-summer and autumn-autumn combinations. By far the highest difference, and the most clearly trustworthy difference, occurs in the autumn-autumn group.

Returning to the problem of the peculiar relationship between winter and autumn production, which has been shown by the distribution of these means to form an exception to the rule that the more closely associated periods have the higher correlation, we may turn back to the results of earlier studies on the relationship between the egg production of various periods of the year in the White Leghorn.

While the deviation of the correlation for the winter and autumn period from the average of all the periods cannot be regarded as statistically significant, it is positive in sign, i.e., the average correlation for the months of these two groups is higher than that of the 12 × 12 permutations of the months of the year. This reminds us of the conclusions already drawn (HARRIS, BLAKESLEE and KIRKPATRICK 1918, pp. 56-69) that there is a more intimate correlation between the egg production of the autumn and winter months (at the beginning and end of the contest year) of the same individual bird than between the egg production of these months and the production of spring and summer months.

On the basis of two-years records from the Storrs contest it has been shown (HARRIS, BLAKESLEE and KIRKPATRICK 1918, pp. 47-49, diagram 6) by the use of a special formula (HARRIS 1909) that the winter months, November, December, January and February, and the autumn months, September and October, show an increase over the theoretical quota of eggs when the annual total egg production rises above the average. The spring and summer months, March, April, May, June and July, show a lower relative contribution to the annual total than might be expected if monthly egg production were proportional to annual egg production, when the total production varies in the direction of an increase above the normal egg production of the flock as a whole. In short, the good layers owe their superiority largely to increased egg production during the autumn and winter months.

Relationship between the condition of the birds with respect to laying activity during individual months of the first year and the annual record of the second year, with special reference to culling for second-year production

The annual record of a bird depends to a very great extent upon continuous laying. In the White Leghorns at least, time of beginning of laying and time of cessation of laying are factors of great importance in determining the annual record of the birds.

In view of this fact it is desirable to determine to what extent the simple criterion of *laying* versus *not laying* in any month of the first year may be used in predicting the annual record to be expected the second year. This method has already been applied to the investigation of first-year production (HARRIS, BLAKESLEE and KIRKPATRICK 1918, pp. 42-44, 49-56). A preliminary note (HARRIS and LEWIS 1921) has given a portion of the results of this section.

While our immediate purpose is the consideration of the second-year production of birds which did and of those which did not lay during given months of the first year, it seems desirable to give the mean for the first-year production of these birds as well. For comparison the results deduced from the data of the INTERNATIONAL EGG-LAYING CONTEST at Storrs during the years 1913-1914 and 1914-1915 (HARRIS, BLAKESLEE and KIRKPATRICK 1918, p. 42) are laid beside those presented here from the Vineland data.

TABLE 9

Mean annual and eleven-months production in first and second year for birds which did and which did not lay during individual months of the first year.

CONDITION OF BIRD IN MONTH OF FIRST YEAR	STORRS DATA FOR FIRST YEAR			VINELAND DATA FOR FIRST AND SECOND YEAR				
	Percent of flock	First-year annual mean	First year 11-months mean	Percent of flock	First-year annual mean	First-year 11-months mean	Second-year annual mean	Second-year 11-months mean
November								
Not laying	40.6	136.8		19.4	144.5		127.8	
Laying	59.4	164.2	156.2	80.6	181.2	168.2	142.7	139.9
Difference		+27.4 17.9%	+19.4 12.7%		+36.7 21.1%	+23.7 13.6%	+14.9 10.7%	+12.1 8.7%
December								
Not laying	38.0	133.6		22.3	142.3		125.9	
Laying	62.0	165.2	155.0	77.7	183.2	169.9	143.8	141.5
Difference		+31.6 20.6%	+21.4 14.0%		+40.9 23.9%	+27.6 15.9%	17.9+ 12.8%	+15.6 11.2%
January								
Not laying	42.5	136.4		20.5	141.6		124.4	
Laying	57.5	165.6	157.2	79.5	182.4	170.4	143.8	137.3
Difference		+29.2 19.1%	+20.8 13.6%		+40.8 23.4%	+28.8 16.5%	+19.4 13.9%	+12.9 9.2%
February								
Not laying	9.9	118.6		5.0	133.6		117.0	
Laying	90.1	157.0	152.1	95.0	176.2	163.6	141.0	131.2
Difference		+38.4 25.1%	+33.5 21.9%		+42.6 24.5%	+30.0 17.2%	+24.0 17.2%	+14.2 10.2%

TABLE 9 (continued)

CONDITION OF BIRD IN MONTH OF FIRST YEAR	STORRS DATA FOR FIRST YEAR			VINELAND DATA FOR FIRST AND SECOND YEAR				
	Percent of flock	First year annual mean	First-year 11-months mean	Percent of flock	First-year annual mean	First-year 11 months mean	Second-year annual mean	Second-year 11-months mean
July								
Not laying..	2.3	72.3		3.1	110.6		92.5	
Laying.....	97.7	155.1	135.4	96.8	176.1	155.5	141.3	123.3
Difference...		+82.8 54.1%	+63.1 41.2%		+65.5 37.6%	+44.9 25.8%	+48.8 34.9%	+30.8 22.0%
August								
Not laying	5.1	89.9		7.2	121.6		99.5	
Laying.....	94.9	156.5	138.3	92.8	178.1	161.2	142.9	128.4
Difference...		+66.6 43.5%	+48.4 31.6%		+56.5 32.5%	+39.6 22.8%	+43.4 31.0%	+28.9 20.7%
September								
Not laying.	23.0	115.0		33.2	147.8		124.3	
Laying....	77.0	164.6	155.4	66.8	187.1	177.5	147.5	135.7
Difference..		+49.6 32.4%	+40.4 26.4%		+39.3 22.6%	+29.7 17.1%	+23.2 16.6%	+11.4 8.2%
October								
Not laying .	54.7	131.9		63.2	156.9		129.8	
Laying.....	45.3	178.9	169.2	36.8	203.5	192.3	157.0	149.7
Difference...		+47.0 30.7%	+37.3 24.3%		+46.6 26.8%	+35.4 20.3%	+27.2 19.5%	+19.9 14.2%

The essential constants are shown in table 9. This gives the percent of the flock which did and which did not lay during the months of the year in which any considerable proportion of the birds did not lay. The average annual production for these birds in the first year of both the Storrs (1913-1914 and 1914-1915) and the Vineland (1917-1918) contests and in the second year (1918-1919) of the Vineland contest are shown. The differences between these annual means for the birds of the two classes and the relative differences, i.e., the ratio of the actual differences to the annual average productions[6] of the flock are also given. While the actual differences in egg production are the data of practical significance, comparison between the three series is facilitated by expressing all of the results in comparable terms.

[6] These are 153.19 eggs for the first year at Storrs, 174.05 eggs for the first year at Vineland, and 139.79 eggs for the second year at Vineland.

The results show material differences in the annual egg record of the birds which do lay and the birds which do not lay in each of the eight months of the year which require consideration. The results for the first-year records at Storrs and the first-year records at Vineland are in fair agreement.

For the Storrs series the birds which lay in any given month show an average annual egg production from 27.4 to 82.8 eggs higher than those which do not lay, or from 17.9 to 54.1 percent. For the first year of the Vineland contest the difference in the egg production of the two groups ranges from 36.7 to 65.5 eggs or from 21.1 to 37.6 percent. Thus the difference in the annual egg production of the birds which do and which do not lay in any given month varies greatly according to the month considered. In both contests the smallest difference in the annual mean of the two groups is shown when selection is based upon the November egg record while the largest difference is that found when the segregation of the flock is based upon the July record. During the months of November, December and January the percentage differences in the annual production of the two groups of birds are higher in the Vineland than in the Storrs series, whereas for the remaining months of the eight considered, the reverse is true. The average percentage difference is 30.4 in the Storrs series and 26.6 in the Vineland series.

Turning to the second-year means we note that for each of the eight months used as a basis of selection the second-year records of birds is higher if they laid in the special month under consideration in the first year than if they did not lay in that month. The differences between the groups amount to about two dozen eggs or more in five of the eight months considered.

The fact that the difference between the second-year means for the laying and non-laying birds is always less than the difference between the first-year means may be attributed to three factors.

1. The average production is lower in the second than in the first year. This factor is obviated when the differences in the two means are expressed as relative differences.

2. Because of the imperfect correlation between the first- and second-year records, selection in the first year is not followed by as large differences in the second year as in the first year.

3. In dividing the birds into two classes,—laying and not laying,—in the first year, we necessarily reduce the number of months of oviposition from twelve to at least eleven for all of the birds of one of the classes. This classification does not, however, directly affect the second year.

To obviate the difficulty mentioned in (3) above we may determine the mean egg production for the eleven other months of the year for the months considered in table 9. The mean production of the eleven months and the mean annual production will necessarily be the same for birds which are not laying in any given month. The mean production for the eleven-month period will, however, be different and somewhat lower than the annual mean for birds which are laying in the months in question.

Consulting the table for the actual constants for the group of eleven months, we note that in the case of the first-year production at both Storrs and Vineland the differences for the birds which do and which do not lay in a given month are smaller than that for the year as a whole. This difference is shown in both the actual numbers of eggs and in the percentage differences.[7]

It seems unnecessary to consider the differences in the eleven-month totals in greater detail. All of the facts are available from table 9. The essential point which requires emphasis here is the fact that in both the Storrs and Vineland series the difference in the eleven-month totals, while not so large as those in the annual totals, are conspicuously different for birds which do and which do not lay in the specific month under consideration. *This shows clearly that the difference in the annual means represent in large part fundamental differences in the egg-laying capacity of the birds and that the observed differences in the annual means are not due primarily to the fact that one-month's egg record is necessarily fixed by the classification into the two groups.*

Turning now to the problem of the average eleven-month production of birds in the second year we find that the difference between the annual mean and the eleven-month mean is in general not so conspicuous as that observed in the case of the first-year production. This is to be expected because of the fact that in the second year the exact equivalent of the egg production of the month of the first year used as the basis of selection is not deduced from the total egg production to obtain the eleven-month average, since the production of the individual months of the first and second year are not (as shown above) perfectly correlated.

We now have to consider an interesting characteristic of these annual averages of birds which are and which are not laying in any of the later months of the year.

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[7] These percentage differences are based upon the annual mean since the eleven-month average differs for each month. The basis used in determining the percent is, however, in reality too large and exaggerates somewhat the apparent difference between comparisons based upon the annual means and the eleven-month means.

For first-year's production it has been shown that the birds which are not laying in any month show a steady increase in the mean annual production as the months approach the end of the year. Similarly, and at first apparently paradoxically, the annual averages for the birds which did lay during these months also show an increase from the earlier to the later final months.

Placing all the records side by side we have the following figures:

TABLE 10

	BIRDS NOT LAYING			BIRDS LAYING		
	Storrs	Vineland I	Vineland II	Storrs	Vineland I	Vineland II
July	72.3	110 6	92.5	155 1	176 1	141 3
August	89 9	121 6	99 5	156.5	178 1	142.9
September	115.0	147.5	124 3	164.6	187 1	147 5
October	131.9	156 9	129 8	178 9	203 5	157 0

This, at first rather paradoxical result, is due to the fact that only the relatively good layers continue to lay into the later months. Thus the average of the birds which are laying is higher in August than in July and higher in September than in August and higher in October than in September. On the other hand the percentage of the flock which is not laying (table 9) increases rapidly from July and August when less than 8 percent of the birds are not laying to September when 23-33 percent of the birds are not laying and to October when 55 to 63 percent of the birds are not laying. As a result of this increase in the percentage of the birds which are not laying in these later months of the year the average quality of the birds of both classes increases rapidly with a resulting higher average annual egg production.

We may now consider as we have in a preceding publication (HARRIS, BLAKESLEE and KIRKPATRICK 1918, p. 50) the results of dividing the flock according to their egg-laying activity in more than a single month of the year. As far as records for individual months are concerned the birds may, if one desires, be divided into alternative classes,—birds which do and birds which do not lay. Birds which do lay may be logically subdivided according to the number of eggs laid. But on the basis of egg records for one month only there is no means of subdividing the class of birds which do not lay in any given month. Nevertheless, it is clear that a bird which laid during September and did not lay during October is a quite different organism from one which laid neither in September nor in October.

This problem has already been considered for the final months of the first-year data of the Storrs contest (HARRIS, BLAKESLEE and KIRKPATRICK 1918, pp. 49-53). We shall here treat both first and second years of the Vineland contest, and shall consider both the initial and the final months of the year.

Table 11 shows the laying activity in the first three months of the pullet year and the mean annual egg production of the first (pullet) and second year.

TABLE 11

First- and second-year production of birds classified according to their activity or inactivity as layers during the first three months of the pullet year.

STATUS OF BIRDS FOR FIRST YEAR	NUMBER OF BIRDS	FIRST-YEAR ANNUAL PRODUCTION	FIRST-YEAR MEAN ANNUAL PRODUCTION	SECOND-YEAR ANNUAL PRODUCTION	SECOND-YEAR MEAN ANNUAL PRODUCTION
Not laying in November....					
Not laying in December...					
Not laying in January...	27	3020	111.9	3047	112.9
Laying in January.....	29	4329	149.3	3643	125.6
Laying in December......					
Not laying in January .	6	816	136.0	843	140.5
Laying in January.... .	24	4259	177.5	3455	144.0
Laying in November......					
Not laying in December....					
Not laying in January...	12	1725	143.8	1456	121.3
Laying in January....	31	5012	161.7	4322	139.4
Laying in December......					
Not laying in January...	46	7327	159.3	5976	129.9
Laying in January......	268	50615	188.9	39185	146.2

Disregarding two of the eight classes in which the number of individuals is too small (6 and 12, respectively) to justify conclusions concerning them, we note that the average first-year production of the six groups ranges from 111.9 eggs for the 27 birds which did not lay during the first three months of the year to 188.9 eggs for those which did lay during these three months. The values of annual egg production for other combinations of laying activity in these three months may be studied by the reader himself from the table.

It is not surprising that birds which are inactive for three months of the year should make a lower annual record during the year than birds which are laying during these months. The point of special interest is the second-year records of these birds. Such records cannot be directly influenced by activity or inactivity during the individual months of the first year.

The last column of table 11 shows that birds which did not lay during the first three months of the pullet year made an average second-year record of 112.9 eggs, as compared with an average second-year production of 146.2 eggs for the 268 birds which laid in each of these three months.

We now turn to the results for groups of two and three months at the end of the pullet year.

Table 12 shows the relationship between the laying activity in the two final months of the first year and the annual averages of first and second years.

TABLE 12

First- and second-year production of birds classified according to their activity or inactivity as layers during September and October of the pullet year.

STATUS OF BIRDS	FIRST YEAR			SECOND YEAR	
	Number of birds	Total annual production	Mean annual production	Total annual production	Mean annual production
Not laying in October					
Not laying in September .	131	18883	144.1	15840	120.9
Laying in September	149	25053	168.1	20500	137.6
Laying in October					
Not laying in September . .	16	2838	177.4	2432	152.0
Laying in September. . .	147	30329	206.3	23155	157.5

For the first-year records the average production increases from 144.1 eggs for birds which are inactive in both September and October to 206.3 eggs for birds which lay in both of these months. For the second year the differences are not so large, but are in full agreement with those for the first year in indicating a progressive increase in the second-year average production as the egg-laying activity of the first year is continued into the two final months of the year. Thus the average second-year production ranges from 120.9 for birds which are inactive in both September and October of the first year to 157.5 eggs for birds which lay some eggs in these final two months of the year.

Table 13 shows the relationship between the laying activity in the final three months of the first year and the average egg production of the first and second years for the 443 birds which completed the first two years of the contest. From this we note that birds which did not lay during August, September or October, had an annual egg record in the first year of 115.3 eggs. Those which laid in August but did not lay during September or October had an average of 150.6 eggs. Those which laid in August and September but did not lay in October laid 169.7 eggs in the year. Those which laid in October and in August but not in September laid 177.4 eggs. Finally, those which laid in August, September and October laid 207.1 eggs. Two of the smaller classes, of three and five birds, respectively, are left out of account here. Thus the annual egg record of the first year depends very largely upon the laying activities in the final three months of the first year.

TABLE 13

First- and second-year production of birds classified according to their activity or inactivity as layers during August, September and October of the pullet year.

STATUS OF BIRDS FOR FIRST YEAR	NUMBER OF BIRDS	FIRST-YEAR MEAN ANNUAL PRODUCTION	SECOND-YEAR MEAN ANNUAL PRODUCTION
Not laying in October			
Not laying in September			
Not laying in August	24	115.3	91.4
Laying in August	107	150.6	127.5
Laying in September			
Not laying in August	5	122.6	102.8
Laying in August	144	169.7	138.8
Laying in October			
Not laying in September			
Not laying in August			
Laying in August	16	177.4	152.0
Laying in September			
Not laying in August	3	171.0	158.3
Laying in August	144	207.1	157.5

The second-year productions parallel very closely indeed those for the first year. Birds which do not lay in August, September or October of the first year average only 91.4 eggs during the second year, those which do not lay in September and October but do lay in August have a record of 127.5 eggs while those which lay in August and September but not in October average 138.8 eggs. Finally, birds which lay in August, September and October of the first year average 157.5 in the second year.

Thus if the birds be divided into groups in the *first year* on the basis of laying *versus* non-laying in the final three months of the year we find that the laying class will average 157.5 eggs in the second year as compared with 91.4 eggs for the non-laying class in the *second year.*

The foregoing results point clearly to an intimate relationship between the time at which egg-laying ceases in the first year and the time at which it ceases in the second year of the bird's life. This relationship is indirectly but unmistakably shown by the fact that the classification of the birds with respect to *laying activity in the initial and in the final months of the* *first* influences profoundly the ***record made by the groups of birds in the second year.***

Approaching the problem of the relationship between the time of the cessation of laying in the two years as directly as it can be on the basis of total production per month,[8] we may classify the birds in a two-fold manner with respect to laying activity for the initial and the final months of the two years.

The results for November egg-laying activity appear in table 14 which shows that 86 birds laid while 77 did not lay in November of the two years. Birds in which the egg-laying activity at comparable periods in the two years differs, comprise 271 which laid in November of the first year but did not lay in November of the second year, and 9 birds did not lay in November of the first year but did lay in November of the second year.

TABLE 14

November laying activities.

Second year

		LAID	DID NOT LAY	TOTALS
First year	Laid .	86 +9.44	271 −9.44	357
	Did not lay	9 −9.44	77 +9.44	86
	Totals.	95	348	443

If the November record of the second year were entirely independent of that of the first year we should expect the birds which did not lay in the second year to represent $\frac{95}{443}$ of both those which did and those which did not lay in November of the first year. Similarly, birds which did not lay in November of the second year should represent $\frac{348}{443}$ of both the group which did and the group which did not lay in the first year. The values with signs show to what extent the absolute frequencies exceed (+) or fall short of (−) expectation on the assumption that the time of beginning of laying in the two years is uncorrelated. Table 15 for December and table 16 for January are formed in the same manner.

In all cases birds which fall in the same class in the two years are more frequent than they should be on the assumption that the time of beginning of laying in the two years is uncorrelated.

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[8] We shall discuss the inter-annual correlations for actual time of beginning and cessation of laying in a subsequent paper in GENETICS.

Turning now to the final months of the year we have the results for August set forth in table 17. The results for September laying activity are shown in table 18. Finally, those for October laying activity in the two years appear in table 19.

TABLE 15

December laying activities

Second year

		LAID	DID NOT LAY	TOTALS
First year	Laid	98 +11.81	246 −11.81	344
	Did not lay	13 −11.81	86 +11.81	99
	Totals	111	332	443

TABLE 16

January laying activities.

Second year

		LAID	DID NOT LAY	TOTALS
First year	Laid	279 +22.35	73 −22.35	352
	Did not lay	44 −22.35	47 +22.35	91
	Totals	323	120	443

TABLE 17

August laying activities.

Second year

		LAID	DID NOT LAY	TOTALS
First year	Laid	371 +10.10	40 −10.10	411
	Did not lay	18 −10.10	14 +10.10	32
	Totals	389	54	443

In all these tables the diagonal frequencies, representing similar conditions in the two years, are far in excess of expectation. Applying the χ^2 test (PEARSON 1900) for goodness of fit, we find

November, $\chi^2 = 7.636$, $P = .005435$
December, $\chi^2 = 9.655$, $P = .022267$
January, $\chi^2 = 34.978$, $P = < .000001$
August, $\chi^2 = 32.097$, $P = .000001$
September, $\chi^2 = 60.859$, $P = .000001$
October, $\chi^2 = 69.628$, $P = .000001$

Here the values of P are taken directly from ELDERTON'S *(1901) table.*

TABLE 18

September laying activities.
Second year

		LAID	DID NOT LAY	TOTALS
First year	Laid	239 +35.88	57 −35.88	296
	Did not lay	65 −35.88	82 +35.88	147
	Totals	304	139	443

TABLE 19

October laying activities.
Second year

		LAID	DID NOT LAY	TOTALS
First year	Laid	90 +39.22	73 −39.22	163
	Did not lay	48 −39.22	232 +39.22	280
	Totals	138	305	443

We note that values of χ^2 increase from November to January and from August to October, thus indicating that the relationship between the laying activity of the two years becomes more intimate as the season progresses at the beginning of the year, and as it draws to a close. The value of P, the probability that deviation systems as great as or greater than those actually observed might have arisen through the errors of random sampling, are so small that it is impossible to consider the status of the birds in the two years as independent. The time of beginning of laying and of cessation of laying in the two years is evidently correlated.

DISCUSSION AND CONCLUSIONS

The purpose of this paper has been to present the results of an investigation of the relationship between the first- and second-year's egg production of the domestic fowl. Published data are reviewed and the results for a flock of 443 White Leghorn fowls completing the first two years of the VINELAND INTERNATIONAL EGG-LAYING AND BREEDING CONTEST are analyzed.

All available series of data comprising reasonably large numbers of birds show a positive correlation between the total egg production of the first and second year. In the present series this interrelationship is measured for the contest flock as a whole by a coefficient of correlation of $r = .539$ to $r = .554$ and by a correlation ratio of $\eta = .559$ to $\eta = .582$, depending on the method of grouping adopted. The conclusions of those who have maintained that there is no sensible relationship between the egg record of the first and second year are, therefore, groundless.

The relationship between the first- and second-year's annual record may be fairly satisfactorily expressed by a straight-line equation.

The correlation within the individual pens is highly variable. This is readily explained as a result of the small number of birds per pen. The coefficients are, however, predominantly positive in sign and on an average slightly larger than that demonstrated for the flock as a whole. It is, therefore, clear that inter-annual correlation for the flock as a whole is due to the individuality of the birds within the pens and not to differentiation of the constituent pens of which the contest flock is composed.

Among poultrymen the opinion has long obtained that birds which lay poorly during the first year may lay more heavily during their second year, or, conversely, that birds which have laid unusually heavily during the first year will make a poor record the second year. *A priori* there might seem to be good morphological and physiological grounds for this belief. It might easily be assumed that unusually heavy laying in the first year would so reduce the number of ovules available for extrusion during the earlier periods of life that fewer would be available for laying the second year. If the problem be considered from the physiological side, it might be assumed that unusually heavy first-year laying would so tax the system of the organism that a heavy second-year record would be impossible. Again, much must depend upon the time of hatching and the time of beginning of laying. If the laying period of the first year be much shortened because of late hatching, it would seem reasonable to suppose that the egg record of the first year might also be unusually small while that of the second year might be unusually large, because of the continuation into the second year of egg-laying activity which normally would have ceased with the autumn of the first year. The results *of the present investigation* show, however, that the mean productions of the first and second years of the individual flocks are correlated. Thus in so far as those contest birds may be considered typical of flocks in general, it appears that birds drawn from a flock of higher first-year's performance may be expected in general to give good results the second year. This may not be universally true, however, and the whole problem of the relationship between first- and second-year flock means deserves further investigation.

The correlations between the monthly productions of the first year and the annual productions of the second year are positive and statistically significant throughout. Numerically the values range from $r = +.167$ to $r = +.435$. The higher values are found in the months of June to October.

The regression of the total production of the second year on the monthly production of the first year is, roughly speaking, linear. It is, therefore, quite feasible to use the record of any individual month of the first year for predicting the annual production of the second year.

The 144 correlation coefficients measuring the relationship between the 12 individual months of the first year and the 12 individual months of the second year have been determined. These are numerically low, but with few exceptions are positive in sign. These correlations have the average value of $\bar{r} = .1585 \pm .0070$. These results show how slender is the support for the assumptions made by geneticists, of the absence of correlation between the production of the first and second laying year. The evidence indicates that the production of every individual month of the second year is correlated with that of every individual month of the first year.

The measures of interrelationship between the several months of the first and second year are not, however, of equal magnitude. This is shown by the fact that, if the true value of the 144 correlation coefficients were biologically the same and the differences in the empirically determined constants due to the errors of random sampling only, we should have a theoretical standard deviation of r of $\sigma_r' = 1 - r^2/\sqrt{443}$. Assuming that the true value of r is given to a very close approximation by the average of the 144 empirically determined coefficients, we find $\sigma_r' = 0.0463$, whereas the value computed from the actual coefficients is $\sigma_r = 0.1242$. Since the assumed true value of r is near the lower end of its possible range, it is clear that other assumptions as to the true value of the inter-annual correlation for periods of one-month duration will on the average give an even greater discrepancy between observation and expectation.

It is, therefore, clear that there are fundamental biological differences in the correlations due to the various permutations of months of the first and the second year of the bird's life.

These differences will be considered in greater detail in a subsequent paper, now in an advanced stage of preparation. In this place, we shall limit our discussion of the point to a consideration of the correlations for the conventional cycles or periods heretofore recognized by geneticists. Grouping the constants according to the conventional winter, spring, summer and autumn "cycles" or periods we find the following deviations of the averages of the correlations between the months of the several periods from the average for the series of 144 correlations (table 20).

TABLE 20

Second year

First year	WINTER	SPRING	SUMMER	AUTUMN
Winter.......	**+ 0575**	**−.0984**	**−.0740**	+.0331
Spring...........	**−.1003**	**+.0674**	+.0030	**−.0506**
Summer..........	**−.0869**	**+ 0872**	**+ 1656**	+ 0358
Autumn.....	− 0308	**− 1206**	+ 0152	**+.2974**

The deviations which may be considered statistically significant (greater than three times the probable error) are printed in heavy type. It is clear that the egg records of homologous periods of the year are more closely correlated than are those of non-homologous periods. The constants show, however, that in White Leghorn fowl it is the autumn rather than the winter periods of the two years which are most highly correlated.

The existence of a correlation between the production of the first and of the second year of the bird's life makes possible the prediction of the average second-year record of birds whose first-year production is known. The economic application of the principle of inter-annual correlation to the culling of the flock for the purpose of increasing second-year production will be discussed elsewhere. In this paper it has been shown that the relationship between the first- and the second-year laying activity is such that the mere classification of the birds into the alternative groups of "laying" and "not laying" in a number of the months of the first year may serve for the separation of groups of birds which will differ materially in their second-year production.

Even greater differences may be obtained in the second year when the birds are grouped according to their activity or non-activity as layers in more than a single month of the first year.

Division into alternative classes with respect to laying activity in the two years indicates that there is a correlation between both the time of beginning and the time of cessation of laying in the first and second year.

A more exhaustive investigation of a number of the problems considered in this paper is now in progress.

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AN EYELESS MUTANT IN *DROSOPHILA HYDEI*

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INTRODUCTION

A new eye structure that shows marked variations in size depending upon the environmental conditions to which the culture is subjected, appeared in my stock of *Drosophila hydei* (Sturtevant) in 1917. Under certain conditions of temperature and moisture the eyes of this mutant stock appear as mere specks or are entirely missing, while the eyes of the same mutant stock under other conditions are very large, in fact indistinguishable from those of the wild type. Between these two extreme types all gradations appear. The mutant has been called "variable."

Cultures of variable subjected to warm dry conditions produce speck-eyed forms in large number; in fact the cultures at times become "pure" for speck eye. When the temperature at which the cultures are kept is reduced, the percentage of flies with large eyes increases. If the culture bottles are well supplied with moist food and kept at a temperature slightly below that of the living room the stock may appear "pure" for the wild-type eye. Cultures kept in a fluctuating laboratory temperature, with food added at irregular intervals, give rise to a wide range from speck to normal-eyed forms with intermediate types. This was the condition under which we were studying the character for a long time after its origin, and until the key to the situation was found, the results of our crosses were most puzzling. By controlling the environmental conditions it can be demonstrated that variable is a recessive Mendelian character which seems to segregate freely from its normal allelomorph. Interest is added to this case since variable is linked with scarlet, another mutant obtained in this stock (HYDE 1915).

Before it became evident that the type of eye shown by the mutant depended upon certain environmental conditions, the results with the different crosses defied interpretation. Attempts to purify the stock by selection of the speck-eyed forms failed; for, after the process had been continued for some time and the stock bottles contained only speck-eyed flies, the next generation would apparently revert to the normal-type eye or give a range of eye sizes. The key to the situation was suggested by a comparison of the records covering a long period of time. The records showed that during the summer the speck condition of the eye was in evidence, while during the winter it was not so likely to appear. The result was explicable on the assumption that temperature was a determining factor in the realization of the character.

CONCERNING *Drosophila repleta* AND ALLIED SPECIES

It has become evident that the term *Drosophila repleta* includes more than a single species. In the American Naturalist for March 1915 STURTEVANT reported a new sex-linked mutant for *Drosophila repleta.* In the same number of the Naturalist I reported scarlet, a new mutant eye color for this same species (HYDE 1915). It is now certain that the *repleta* with which STURTEVANT worked and mine are two different species. They resemble each other so closely that despite the fact that I had bred both forms for a long time the difference was not discovered until many attempts at crossing had failed. Failure to cross is admitted to be sufficient ground for separating different species. Closer study has shown that the two differ also in certain morphological characters. In STURTEVANT'S type the lower side of the thorax including the basal segments of the legs is distinctly smoky in color, while in my type these parts are yellowish with the merest hint in some of a smoky color. In STURTEVANT'S type the yellowish segmental bands on the upper part of the abdomen are intersected by the alternating dark bands as they extend to the sides of the abdomen, resulting in a demarkation on the side of the animal of a row of light spots. In my type these spots are only faintly in evidence. I have found STURTEVANT'S type in New York and Baltimore, but not in Indiana. My type is very common about fruit stores and orchards in Indiana, but so far I have not found it in Baltimore. There is still another type of *Drosophila repleta* that differs from the two types under discussion. It is characterized by rather large light spots on the sides of the abdomen. I have taken it during two different summers in the open woods in Indiana. It is very hard to breed in captivity. There would seem then to be at least three or four different species of this fly passing under the name *Drosophila repleta.* I have found three light males, of STURTEVANT'S mutant type taken at Arlington, Maryland, but have not as yet determined the inheritance of the character.

Since this was written STURTEVANT'S catalog of the North American Drosophilae has appeared, in which these flies are described (STURTEVANT 1921). STURTEVANT'S mutant is named *Drosophila repleta.* The mutant scarlet, previously reported and the "variable" of this paper belong to a new species called *Drosophila hydei.* The third form is probably a new species named by STURTEVANT, *D. mulleri.*

ORIGIN AND DESCRIPTION OF VARIABLE

Variable was first noted January 13, 1917, among the offspring from a cross of the mutant scarlet to wild stock in which the behavior of the mutant scarlet in back-crosses was to be tested (bottle 10, table III, Proceedings Indiana Academy 1917). In a total of about 100 flies, twenty were found with eyes that varied in size from mere specks (or in fact no eyes), to eyes of normal size and shape. Since both scarlet and wild-type eyes were similarly affected it is highly probable that these mutants do not represent the first mutation of the gene. It is impossible to state whether the change originated in the wild stock or in the mutated scarlet stock.

The ommatidia of the affected eyes are missing from the outer border of the eye, leaving a light brown band encircling the reduced eye. This holds for eyes down to about half size. Below this stage of reduction the eyes are often irregular, the light area is apparently carried toward the center where the few ommatidia protrude as an irregular mass from a somewhat depressed area. When reduced to a speck, this lies slightly posterior to the center of the normal eye. The small-eyed animals have an arrow-headed appearance which is in marked contrast to the wild flies with their large eyes and blunt heads.

Speck eyes were selected and inbred for a few generations as were the flies with eyes about half normal size, but both have given descendants with practically the same range of forms. The stock has apparently changed, however, in another particular, for a comparison of the descendants with the mutants that originally appeared will show that the eyes of each affected individual are more symmetrical in size and shape. The eyes of the first twenty mutants were very asymmetrical. The eye color of the mature wild-type *repleta* is very dark and will be referred to throughout the paper as mahogany.

EARLY HISTORY OF THE BEHAVIOR OF VARIABLE IN INHERITANCE

A small amount of data is given here to show the type of complicated results obtained before the key to the situation was found. The twenty flies discovered on January 13, 1917, were made up in three different cultures, and to my great surprise only normal-eyed flies appeared among the descendants until April 30 when a count yielded 2 males and 4 females with eyes about one-half normal size, 15 normal-eyed males, and 15 normal-eyed females. On May 3, there appeared 45 with normal eyes, and 15 flies with eyes one-half normal size. The small-eyed forms were selected and produced 466 normal flies and 32 with eyes that varied in size from speck to normal. The small-eyed flies were again selected, and in the summer of 1917 a count from this culture gave 147 females, and 142 males with eyes varying from one-half normal size to eyes entirely missing. Crosses were made by my students with this stock during the winter of 1917-1918. Ignorance of the fact that temperature was a determining factor in the realization of the character, led us at the time to ascribe our conflicting results to lack of purity of the stock.

Crosses between the wild stock and variable, made on March 2, 1918, gave the following results: The wild-stock ♂ mated to the scarlet variable ♀ gave more than 400 offspring with eyes like those of the wild type. These when inbred produced 1554 mahogany normal, 35 mahogany variable, 331 scarlet normal and 38 scarlet variable.

The reciprocal cross in which the scarlet variable ♂ was crossed to the wild-stock ♀ yielded about 400 sons and daughters with eyes characteristic of the wild type. These when inbred produced 390 mahogany normal, 8 mahogany variable, 81 scarlet normal and 14 scarlet variable.

These crosses give a grand total of 1944 mahogany to 464 scarlet, a ratio of 4.3 to 1. The ratio usually obtained on crossing scarlet to the wild type is 3 wild type to 1 scarlet. It seems likely that variable has in some way modified the ratio.

There are 2356 normal-eyed flies and 105 variables, a ratio of 22 to 1, a result we can understand in part when we know that the temperature can shift such a result within very wide limits.

BEHAVIOR OF VARIABLE UNDER A CONTROLLED TEMPERATURE

The study was carried forward from this point in light of the fact that eyeless flies were more likely to appear at high temperatures than at low temperatures. During the summer of 1918 the speck-eyed form had appeared in large numbers. On October 5, there appeared in a culture bottle that had been made up on May 14, 30 flies with eyes that varied from one-half the normal size to flies with mere specks for eyes. Several cultures were made from this bottle and placed in a rudely constructed warm chamber. The temperature fluctuated between 25° to 30° C. Speck-eyed flies emerged by the hundreds. In fact the cultures were pure for this character, while the flies that emerged from the control bottles, kept at a reduced room temperature during October and November, had eyes indistinguishable from the normal type. This showed that temperature was at least one deciding factor in the realization of the new character.

That moisture plays some part in the expression of the character would seem to be indicated by the fact that invariably an increasing number of flies with reduced eyes appear as the cultures become dry. Moreover the speck condition becomes intensified in dry cultures. It frequently happens that the first flies to emerge from pure variable cultures that have been kept at a relatively high temperature, have eyes larger than those that emerge later. In fact the first flies that emerge may be indistinguishable from normal while later only speck-eyed forms appear. This is shown in table 1. The obvious interpretation is that development has been accelerated by the temperature so that the plastic moment in the life of the fly is passed before the temperature has brought about any change in the moisture conditions of the culture. Many changes are taking place in a fermenting mass of banana exposed to a temperature of 25° to 30° C, so that one can not be sure from the evidence at hand that temperature exerts its influence through a change in moisture. We can only assert that the eyeless condition is realized under warm dry conditions.

LINKAGE OF VARIABLE AND SCARLET

With a stock that was pure for variable as shown by a number of tests and with the fact established that the character could be controlled by subjecting the cultures to the proper temperatures, the behavior of scarlet and variable in the same cross was determined. Wild-stock virgin females, whose history was known, were mated to the double recessive scarlet speck-eyed males and kept at room temperature. All of the hybrids were like the wild type. These were back-crossed to the double-recessive parent stock. The cultures were kept in the warm chamber with the result shown in table 2. This table lists the results from three such tests. The data would seem to indicate that the full temperature effects were not realized, but it is evident that there is linkage between scarlet and variable. No mahogany variable flies result from this combination, which is explicable on the assumption that the characters do not cross over in the male.

TABLE 1

This table shows the history of two culture bottles of pure variable, that were well supplied with food and kept at a high temperature. Note that the first flies to hatch have larger eyes than those that hatch later

CULTURE NUMBER	DATE EXAMINED, NOVEMBER, 1918	NUMBER OF FLIES	CONDITION OF EYE
1	9	10	Eyes normal size
	14	9	Slight reduction
	16	22	One-half size
	20	150	One-half size to mere specks
	22	100	One-half size to minimal
2	9	14	Slight reduction
	14	19	Slight deduction
	16	15	Three-fourths size
	20	100	About one-half size
	22	35	One-half size—a few minimal
	28	30	One-half size to minimal

The relation between scarlet and variable was also studied in the reciprocal cross in which the hybrid ♀ was back-crossed to the double-recessive scarlet variable ♂, under the same conditions as in the above test. From this combination all four classes appear as in table 3. This table lists the results from three separate tests. These results are consistent in showing that variable and scarlet are linked and that there is crossing over in the female and none in the male. The crosses, however, do not yield the ratios which the formula demands. This is probably due to lack of environmental control, although genetic factors may be involved. Certain difficulties are met in breeding this fly which make some of the problems here encountered hard to bring under control.

Tables 2 and 3 are compiled from records which show the sex ratio in each of the different classes to be near equality. The detailed records also show that in so-far as the distribution of the characters from the back-crosses are concerned it makes no difference whether the double recessive entered the hybrid from the male or female.

TABLE 2

Back-cross. Offspring from scarlet eyeless ♀ × hybrid ♂ (Wild × scarlet eyeless). Cultures kept in warm box. The tables list the totals from three separate tests. The sex ratio for each of the different classes was near equality.

CLASSES.	NORMAL MAHOGANY	NORMAL SCARLET	MAHOGANY VARIABLE	SCARLET VARIABLE
Test 1......	1023	443	0	412
Test 2......	16	3	0	2
Test 3.....	35	11	0	11
Total..	1074	457	0	425

TABLE 3

Back-cross. Reciprocal of 2. Offspring from hybrid ♀ × scarlet eyeless ♂ (wild × scarlet eyeless). Cultures were kept in warm chamber. The sex ratio for each of the different classes was near equality. The data are compiled from three separate tests.

CLASSES	NORMAL MAHOGANY	NORMAL SCARLET	MAHOGANY VARIABLE	SCARLET VARIABLE
Test 1.....	876	634	103	271
Test 2. .	113	60	11	24
Test 3. . .	63	22	2	7
Total ..	1052	726	116	302

STAGE IN THE LIFE CYCLE OF THE FLY AT WHICH THE ENVIRONMENTAL INFLUENCE IS EXERTED

The following experiment demonstrates that the environmental influence which determines whether the eye of the homozygous variable shall be large or small is exerted after the egg is fertilized and deposited. On January 1, 1919, 100 speck-eyed flies were taken from a pure culture of variable scarlet from the warm box. The selected flies were kept warm and were permitted to lay their eggs on filter paper to which a small piece of banana had been added. Every twenty-four hours the banana with the eggs was placed in a separate bottle with fresh food and returned to the warm box to develop. On the eighth day of the test the eggs were divided into two lots, one lot was returned to the warm box and one was placed on the window ledge where the temperature at this time of year was rather cool.

A comparison of the flies that emerged on the corresponding dates as given in tables 4 and 5 will show that those subjected to the warm temperature had defective eyes, while the eyes of those that developed at the reduced temperature were like the wild type. These results are not to be explained in terms of viability. It is clear that temperature is a determining factor in the realization of the speck-eyed form, and that the influence is exerted after the egg is fertilized and deposited.

Table 4 brings out the fact that moisture plays a part in the realization of the speck-eyed flies. The first flies to hatch from the warm cultures have eyes larger than those that hatch later, that is after the culture begins to dry. The warm box in which the eggs were kept isolated during the first five days in January dropped somewhat in temperature due to extremely cold weather. On January 6, 1919, the eggs were transferred to a newly constructed chamber that was kept more nearly uniform at 28° C. The effect is shown in table 4, cultures 12 to 15. It would seem that when development is accelerated as a result of exposure to high temperature, the flies are likely to have large eyes, as in these cultures they approximate the normal size. If delayed, as in culture 8, until a certain drying effect is produced, the first flies to appear are likely to be of minimal size.

CROSSES INVOLVING DIFFERENT COMBINATIONS OF SCARLET AND VARIABLE AND THEIR NORMAL ALLELOMORPHS UNDER DIFFERENT TEMPERATURE CONDITIONS

A number of crosses have been made in order to determine whether or not the ratios are modified as a result of keeping the parents at one temperature and subjecting their offspring to another. The result is not conclusive as bearing on this point as the ratios obtained approximated those we had obtained during warm summer temperatures. The data, however, are given as substantiation of the claim as to the behavior of the character in inheritance. In some of these combinations flies genotypically eyeless but phenotypically normal were used as indicated in the table headings.

TABLE 4

The type of eye that results on subjecting the fertilized eggs of scarlet variable to a warm temperature. Compare with table 5.

DATE OF ISOLATION OF EGGS JANUARY, 1919	BOTTLE NUMBER	NUMBER OF DAYS OF INCUBATION, NUMBER OF FLIES EMERGED AND CONDITION OF EYES											
		Days	Flies	Eyes	Days	Flies	Eyes	Days	Flies	Eyes	Days	Flies	Eyes
1	1	16	35	$\frac{3}{4}$							38		
2	2	15	22	$\frac{3}{4}$							37		
3	3	14	20	$\frac{3}{4}$							36	10	m
4	4	13	10	$\frac{3}{4}$	16	7	$\frac{3}{4}$	23	4	m	35		
5	5				15	4	$\frac{1}{2}$	22	11	m	34	5	m
6	6				14	0		21	1	m	33	20	m
7	7				13	0		20	0		32	10	$\frac{3}{4}$
	7a				13	0		20	1	m	32	20	m
8	8				16	0		19	0	m	31	10	m
9	9				15	0		18	12	m	30	3	m
10	10				14	56	$\frac{1}{2}$	17	28	$\frac{1}{2}$m	29	13	m
11	11				13	0		16	0		28	0	
12	12				12	9	$\frac{1}{2}$	15	15	$\frac{1}{2}$+	27	0	
13	13				11	0		14	25	$\frac{1}{2}$	26	25	m
14	14				10	1	$\frac{1}{2}$	13	20	$\frac{3}{4}$	25		
15	15				8	0		11	15	N−	23		

N = normal
N− = slightly less than normal
$\frac{3}{4}$ = $\frac{3}{4}$ normal size
$\frac{1}{2}$ = $\frac{1}{2}$ normal size
$\frac{1}{2}$ − = less than $\frac{1}{2}$ normal size
m = minimal, i.-e., mere specks

TABLE 5

The type of eye that results on subjecting the fertilized eggs of scarlet variable to a reduced temperature. Eggs laid by the same parents as in table 4. These eggs give rise to flies with normal eyes while the eggs used in table 4 give rise to defective eyes.

Date of Isolation	January, 1919									
	8	9	10	11	12	13	14	15	16	17
Number of flies emerged	21	8	13	1	1	0	50	10	30	4
Condition of eyes.	Normal	Normal	Normal	Normal	Normal	Normal	Normal*	Normal	Normal	Normal

*A few flies with eyes slightly smaller than normal.

Wild-stock ♀ × scarlet variable ♂ with normal eye

This combination made November 13, 1918, and kept warm, gave more than 100 flies like that of the wild type. Virgin F_1 flies were mated in pairs and returned to the warm box with result given in table 6.

TABLE 6

F_2 from wild-stock ♀ × scarlet normal-eye ♂ from eyeless stock. The cultures were kept in the warm chamber.

CLASSES	MAHOGANY NORMAL		SCARLET NORMAL		MAHOGANY VARIABLE		SCARLET VARIABLE	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Total	281	301	53	84	8	4	8	7

Wild-stock ♀ × variable scarlet ♂ with very small eyes

The wild-stock ♀ crossed to the variable scarlet ♂ with very small eyes, made at the same time as the cross in the previous experiment, gave in F_1 over 100 flies all of the wild type. These inbred produced in the F_2, classes given in table 7.

Normal-eyed ♀ from variable scarlet stock × wild-stock ♂

Normal-eyed ♀ from variable scarlet stock × wild-stock ♂ mated November 12, 1918, gave 75 wild-type flies. These inbred and kept warm gave in F_2 the different classes as shown in table 8. The F_1 was mated December 19, 1918.

TABLE 7

F_2 from wild-stock ♀ × variable scarlet ♂ with minimal eyes. Cultures kept in warm chamber.

CLASSES..............	MAHOGANY NORMAL		SCARLET NORMAL		MAHOGANY VARIABLE		SCARLET VARIABLE		REMARKS
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
	9	28	6	3	0	0	0	0	
	40	34	6	11	1	0	3	1	
	70	67	8	8	0	0	1	0	
									147 normal 14 speck
Total...........	119	129	20	22	1	0	4	1	

TABLE 8

F_2 from normal-eyed variable scarlet ♀ × wild-stock ♂.

CLASSES	MAHOGANY NORMAL		SCARLET NORMAL		MAHOGANY VARIABLE		SCARLET VARIABLE	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
Total	44	41	9	10	1	1	0	1

Wild-stock ♀ × minimal-eyed scarlet ♂

The wild-stock ♀ was crossed to 6 minimal-eyed scarlet males November 12, 1918, and kept at cool room temperature. On December 31, 1918, over 100 wild-type flies appeared. These were mated in pairs and one set kept in the warm box and one in the cold.

The F_1 was mated December 31 and produced the classes shown in table 9.

TABLE 9

F_2 from wild-stock ♀ × speck-eyed scarlet ♂.

TEMPERATURE CONDITIONS	CLASSES.......	MAHOGANY NORMAL	SCARLET NORMAL	MAHOGANY VARIABLE	SCARLET VARIABLE
Warm...............	Total	801	189	9	42
Cool................	Total	170	40	3	7

Normal-eyed scarlet ♀ from pure minimal mass culture × wild-stock ♂

This cross kept at cool room temperature gave 85 all wild-type flies. A pair of the F_1 mated January 2, 1919, produced on January 30: normal mahogany 168; normal scarlet, 12♂♂, 13♀♀; variable mahogany, 3♂♂, 1♀♀; and variable scarlet, 6♂♂, 3♀♀.

Wild-stock ♀ × normal ♂ from minimal

A wild-stock female was crossed with a normal male from minimal November 13, 1918, and kept at cool room temperature. The F_1 gave 150 all wild type. Pairs were subjected to warm and cool conditions and yielded as shown in tables 10 and 11.

TABLE 10

F_2 from wild-stock ♀ × normal ♂ from variable stock kept in warm box.

CLASSES............	MAHOGANY NORMAL	SCARLET NORMAL	MAHOGANY VARIABLE		SCARLET VARIABLE	
	Both sexes	Both sexes	♂♂	♀♀	♂♂	♀♀
Total......	2110	456	7	7	18	26

TABLE 11

F_2 from wild-stock ♀ × normal ♂ from variable kept at cool room temperature.

CLASSES.	MAHOGANY NORMAL	SCARLET NORMAL	MAHOGANY VARIABLE	SCARLET VARIABLE
Total.. . .	100	22	1	0

OBSERVATIONS ON THE STOCK CULTURES OF VARIABLE

Among the normal-eyed descendants of the original bottle from which the 20 variable flies appeared there was found on April 7, 1917, a scarlet-eyed female with eyes about one-half normal size. This culture was under observation for the inheritance of a suspected wing mutation. This fly was mated to a somatically normal-eyed scarlet male from the same culture. The F_1 generation gave: 35 normal-eyed ♂♂, 40 normal-eyed ♀♀, 9 variable ♂♂ and 15 variable ♀♀.

The variable stock was established by selecting variable eye from the flies just listed. In June they produced 147 females and 142 males pure for variable. The eyes ranged from one-half the normal size to eyes almost entirely missing.

TABLE 12

Summary of the combinations made and classes realized, arranged to show that scarlet and variable are linked and that the characters do not cross over in the male.

SET OF EXPERIMENTS	P_1 COMBINATION	F_1	F_2 CLASSES			
I and III			Mahogany normal	Scarlet normal	Mahogany variable	Scarlet variable
	Variable scarlet ♀ × normal mahogany ♂	All wild type	384	109	18	13
			761	161	15	22
			793	170	20	16
			85	19	2	1
			127	20	1	3
			168	25	4	9
Total..........			2318	504	60	64
	Normal mahogany ♀ × variable scarlet ♂	All wild type	164	25	2	5
			226	56	6	9
			582	137	12	15
			248	42	1	5
			801	189	9	42
			170	40	3	7
			2176	479	15	51
			100	22	1	0
Total..........			4567	990	49	134
II	P_1		F_1 CLASSES			
	Back-cross hybrid ♀ × variable scarlet ♂		876	634	103	271
			113	60	11	24
			63	22	2	7
Total..........			1052	716	116	302
	Back-cross variable scarlet ♀ × hybrid ♂		1023	443	0	412
			16	3	0	2
			35	11	0	11
Total			1074	457	0	425

The normal-eyed flies of April 30 were made up in mass culture and produced 466 normal-eyed flies and 32 with eyes varying from almost none to normal.

A female from the purified variable stock that had mere specks for eyes was mated to wild-stock males on May 28, 1917. This gave during June 21st to 24th, 27 sons and 22 daughters, all with wild-type eyes.

These inbred produced in the next generation: 384 normal mahogany, 109 normal scarlet, 18 variable mahogany, 13 variable scarlet.

During the fall and winter of 1917-1918 the inheritance of variable was studied in different combinations of back-crosses, but most disturbing ratios appeared. An examination of the stock bottles showed that they had apparently reverted to normal. I attributed this at the time to contamination and in the spring of 1918 commenced once again to purify the stock by selection. In this I was apparently successful, for in the summer only speck-eyed forms were to be found in the cultures.

October 5, 1918. From bottle made up May 14, 30 flies with eyes fairly uniform, about one-half the normal size. This bottle was kept warm and small amounts of banana added from time to time. October 14, all very small eyes. October 21, 8 very small eyes. October 28, many flies with eyes one-half size to minimal. October 30, many one-half size and larger. November 4, many one-half size and larger; 2 minimal.

The bottle was now placed near the window in the cold and on December 12 all the flies had eyes of the wild-type size. The bottle was placed again in the warm chamber and on January 9, 1919, all the flies had very small eyes. A bottle of variable produced on November 5, 1919, 2 normal, later 10 normal and 18 normal. It was then placed in the warm box and on December 7, 20 flies, with eyes one-half size and less, appeared, later 21 flies all with mere specks appeared. A bottle of minimal-eyed forms was made up October 8 and kept at cool room temperature. It produced: November 11, 36 normal. November 13, 12 normal. November 15, 27 normal. November 27, 27 normal and one slightly less than normal.

During the fall and winter of 1918-1919 experiments were carried out as recorded in tables 2 to 9. The key to the production of eyeless was evident. It could be made to appear in warm dry conditions.

A bottle made up from minimal-eyed forms on October 8, 1919, was kept at cool room temperature until November 4, 1919. It was then placed in the warm box. The larvae were good size when placed in the box. November 14, 5 normal. November 15, 10 normal. November 16, 16 normal. November 18, 13 less than normal. November 20, 4 one-half and less than normal. November 22, 3 one-half and less than normal. November 26, 4 one-half and less than normal.

Two bottles kept at cool room temperature and dry (that is, no great excess of food was added). Made up October 19, 1919. Bottle 1 produced: November 27, 36 slightly less than normal. December 10, 25 slightly less than normal. Bottle 2 produced: November 27, 9 slightly less than normal. December 10, 40 slightly less than normal.

The stock at present is being subjected to a more exact environmental control.

RECORD OF TWO EYE MOSAICS

A mosaic male with the left eye scarlet and the right eye of wild-type color was found on March 12, 1919, from the descendants of a cross between the wild-stock male and normal scarlet variable female. The anomaly did not recur among the descendants and the inheritance was such as to show that the male was genotypically mahogany.

The second mosaic was a female that appeared October 19, 1920, with the lower third of the right eye covered with an irregular patch of scarlet; the remainder of the eye was mahogany, as was the left eye. This mosaic came from a mixed culture of mahogany and scarlet. No variables were present at this time. This fly was heterozygous; for, when mated to the scarlet male she gave 101 scarlet and 84 mahogany offspring.

DISCUSSION

A few cases in which an environmental stimulus caused marked phenotypic differences have been reported. In Primula BAUR (1911) showed that the plant transmitted the ability to produce red flowers at 15°C and white ones at 30°C. HOGE (1915) found that with a certain mutant of *Drosophila melanogaster*, reduplicated legs appeared in large numbers when subjected to a temperature of 9° to 10°C. ROBERTS (1918) reports that the length of the wings of vestigial is greatly modified by differences in temperature of 4° to 5°C. MORGAN (1915) has shown that the development of the abnormal abdomen in Drosophila is dependent upon moisture. KRAFKA (1920) in a very carefully planned series of experiments has recently shown that the number of facets that appear in the bar-eyed mutant of Drosophila is an expression of the temperature to which they are subjected at certain stages of development.

SUMMARY

A mutant, here called variable, of *Drosophila hydei* (Sturtevant) appeared in the same stock from which the mutant scarlet originated. These

are the only two mutants thus far reported in this species and they show linkage with no crossing over in the male.

The new character may appear in two different forms: (1) Full-eyed, indistinguishable from the normal eye, and (2) less than full-eyed, varying to mere specks.

The expression of the character, when homozygous, depends upon environmental conditions. Cultures of variable kept warm and dry produce speck-eyed forms in large numbers, while the cultures of variable kept cool and moist give rise to flies with normal-sized eyes. Temperature seems to exert its influence through its drying effect; for there is a marked tendency for dry cultures to produce speck-eyed forms. Newly made cultures of variable, subjected to a high temperature, in which the rate of development is greatly accelerated, are likely to produce eyes that approach the normal size.

There is an optimum temperature point beyond which development is so rapid that the eggs hatch while the culture is still wet even though at a high temperature. The flies that thus arise have eyes approaching normal size. On the other hand the temperature may be lowered and the culture become dry so that there is a tendency for a few small-eyed flies to appear even at a low temperature. A culture of variable kept moist and cold produces full-eyed forms, but when kept warm and dry, speck-eyed forms. It is clear that in so far as the expression of the character is concerned the influence is exerted after the egg is fertilized and deposited.

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LINKAGE RELATIONS OF THE SEX-LINKED CHARACTERS IN DROSOPHILA OBSCURA[1]

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INTRODUCTION

The cytological researches of METZ (1914, 1916) showed that there was considerable difference between the chromosome groups of various species of Drosophila. It appeared that here was a good opportunity to test and extend the chromosome theory of heredity. The problem of working out the homologies of the chromosome groups in a genetic way to show the possible derivation of the different types of groupings is important. STURTEVANT (1921) has recently shown that there is near, if not complete, parallelism in the X chromosomes of *D. melanogaster* and a very similar species, *D. simulans*. By crossing mutants of *D. simulans* to mutants of similar appearance in *D. melanogaster* (*ampelophila*), STURTEVANT (1921) has been able to show conclusively that many of these are actually identical in that they act as allelomorphs and that their location in the chromosome agrees as to sequence and approximately as to "distance." These two species are very close to each other taxonomically and probably have the same chromosome complex.

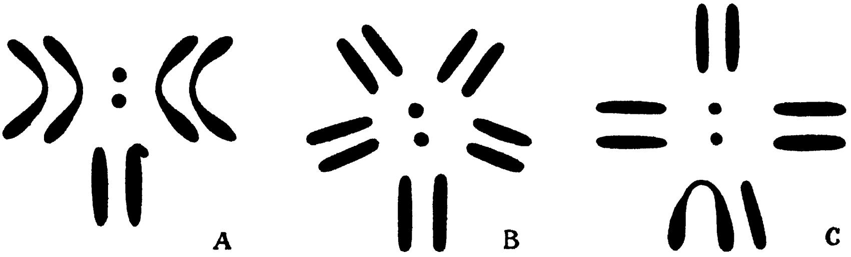

FIGURE 1.—Diagram of male chromosome groups of three species of Drosophila: A, *D. melanogaster;* B, *D. virilis; C, D. obscura* (after METZ).

Drosophila melanogaster, *virilis*, and *obscura* have somewhat similar chromosome groups which are shown for comparison in figure 1. These chromosome diagrams are based on the cytological study of METZ (1916) and show the male groups for the three species. The sex chromosomes are represented at the bottom of each figure. The study of *obscura* was undertaken with the hope that ultimately enough genetic evidence might be accumulated to show how the chromosome map in this species was related to that of the other two types on which extensive genetic data are available. The question of determining homology of chromosomes by comparison of similar mutants in species where allelomorphism cannot be determined by interspecific crosses, will be discussed in a later section. Our knowledge is still too limited to allow comparisons to be carried very far. However, the evidence presented in this paper justifies the belief that certain regions show parallelism and, furthermore, that a chromosome rearrangement has taken place.

An interesting feature about *D. obscura* is that the sex chromosomes are dimorphic, the X being quite long as compared with the Y. The Y is a rod similar in size to that in *D. melanogaster*, which differs in having a short hook. The long V-shaped chromosome, which is the X in *obscura*, contains about two-fifths of the chromatin of the haploid group at the metaphase stage. Whether the genetic phenomena would show any correspondence to this morphological length was an interesting question which it is now possible to answer. The present paper will be concerned largely with a description of sex-linked mutations and the working out of their linkage relations. This group shows such a large amount of crossing over, as to constitute a unique feature of the genetics of this species, and thus offers another confirmation of the chromosome theory of heredity.

STOCKS AND METHODS

Drosophila obscura agrees fairly closely in its general anatomical features with *D. melanogaster*. It is somewhat larger and is much darker, being grayish black rather than yellowish brown in color. The males have two sex combs on each front leg instead of only one, as in *melanogaster*, and in *obscura* the male abdomen is distinctly colored (red) by the testes. A description may be found in STURTEVANT'S (1921) monograph.

The work was started in the summer of 1919, using a wild-stock culture from the Pacific Coast which Dr. C. W. METZ had obtained several years earlier. This stock of *D. obscura* has now been kept going in the laboratory by means of mass culture transfers for upwards of five years, and during this time it has shown no noticeable decline in vigor or productivity. The species breeds more slowly than *D. melanogaster*, and takes fourteen to twenty days for a generation, depending on the temperature. The stock must have passed through at least eighty-five generations while under laboratory conditions. Later in the same summer (1919) another wild stock from Berkeley, California, was sent to Dr. METZ by Prof. E. B. BABCOCK, and this stock has also been used extensively.

The procedure adopted in breeding this species consists of making up matings, usually in pairs, and keeping the flies for two or three days in a vial before transferring to a half-pint bottle containing banana agar, seeded with yeast (BRIDGES 1920). In the vial, the females mature and are ready to lay their eggs as soon as they are transferred to the culture bottle. These bottles are kept until hatching in an incubator regulated to about 25°C. It is desirable for linkage experiments that all cultures be reared at nearly the same temperature, since the amount of crossing over perhaps varies with change of temperature, as PLOUGH (1917) has demonstrated for *D. melanogaster;* however, the effect of change of temperature upon linkage has not yet been determined for this species. The count from a bottle may be continued with safety for twelve or fourteen days, but should be discontinued before the succeeding generation appears. Under ordinary conditions a pair may be expected to produce 150 to 250 offspring.

RESULTS

A summary of the results is given below and is followed by a brief statement and discussion of each point in the succeeding sections.

(1) *Drosophila obscura* mutates with sufficient frequency for genetic study.

(2) Non-disjunction of the sex chromosomes was not found.

(3) Crossing over between sex-linked characters is very frequent and genetically the X chromosome is over 170 units long.

(4) The number of linkage groups equals but does not exceed the haploid number of chromosomes.

(5) Some of the mutations seem to be identical with those in other species.

(6) The X chromosome of *obscura* appears to include a region not present in the X chromosome of *melanogaster.*

Mutability of Drosophila obscura

Drosophila obscura has produced over 40 mutants, a sufficient number to make it a promising species for genetic work. There have also appeared numerous variations which, although they doubtless have a genetic basis, yet are of little value in the study of heredity for one reason or another. Their most common fault is that the character may overlap the normal so frequently as to make an accurate classification impossible. Many mutants have a high degree of sterility, and still others only manifest themselves by a lethal effect, that is, individuals of a certain constitution do not survive. Mutation does not always produce extreme variations. The ones useful for genetic analysis are the ones sufficiently different to permit accurate classification.

Mutants have been found more frequently in certain species than in others. Within a given species mutations occur oftener in certain loci than in others. These same loci may mutate frequently in other species. However, loci which are very mutable in one species may not be so in another, and the second species may show mutable loci not known in the former. The number of mutants that are found depends largely on the number of individuals observed, and has no noticeable correlation to season. The proportion of mutant genes which are dominant to their normal allelomorphs in *melanogaster* has been estimated as being about one in twelve, but it exceeds this in *obscura*, being about one in three, thus far.

Absence of non-disjunction

One of the proofs of the chromosome theory of heredity rests on the phenomenon of non-disjunction of the sex chromosomes of *D. melanogaster* (BRIDGES 1913, 1916). At the suggestion of Dr. C. W. METZ, I looked for non-disjunction in *D. obscura*. As many as 309 cultures that would have revealed non-disjunction if it had occurred were raised and inspected, but it was not found. If non-disjunction ever occurs in this species, it is probably less frequent than in *D. melanogaster*.

Frequent crossing over in the sex-linked group

The genetic length of the chromosome is obtained by adding together the percentages of crossing over between adjacent loci, and counting one percent as one unit of map distance. This gives a total of about 170 units for the X chromosome of *D. obscura*. Further data may very likely show that this value is only approximately correct. The amount of crossing over that occurs in the X chromosome of *D. obscura* far exceeds that in any other known chromosome. The question arises as to what is meant by a linkage group when such a high percentage of crossing over may be obtained. According to the chromosome theory of heredity, a linkage "group" consists of all the genes in one chromosome. Two genes, far distant from each other in the chromosome, might show free separation, but would still show very definite linkage to intermediate genes. Two genes which are far enough apart to have a map distance of 100 or more units will not be separated in every gamete but will only show a separation half the time, or even slightly less frequently. This may seem at first a paradox. The apparent contradiction is at once eliminated when one realizes that two breaks, or crossovers, may occur between two loci and, according to the method used, will be counted as two crossovers although no separation of the loci is produced. The frequency of separation does not go above 50 percent, even where the total amount of crossing over may mount up to 170 percent. The reason for this is that a single crossover between two genes will separate them, while a double crossover will result in an exchange of an intermediate group of genes that will leave the two in question still together. That the amount of separation between any two genes does not exceed 50 percent (even where the amount of crossing over based on summation is calculated as far greater than this) is due to the fact that the relative frequency of double crossing over increases as the distance becomes longer. In a chromosome as "long" as the one worked with here, double crossing over may become as frequent as single crossing over, so that it cannot be determined from inspection of the crossover classes, which are single and which double. In some of the early three-point crosses, there was so little difference in size between the double, the two single, and the non-crossover classes that the sequence of the loci could not be determined. Discovery and use of other mutants which were intermediate in position helped to settle the difficulty. A four-point cross was made in which it was possible to determine the sequence of the four loci. This order has been repeatedly confirmed by many crosses made to determine the position of new genes in the group. A detailed discussion of the way in which the positions of the various sex-linked genes were determined in working out the X-chromosome map will be found in a later section. The amount of crossing over between the various loci, when summated, totals, as stated, 170 percent, which exceeds the genetic length of any other chromosome so far studied, by at least 50 units.

Correspondence in number of chromosomes and linkage groups

If the chromosomes are the bearers of the hereditary factors, and if a group of linked genes is composed of the genes borne by one chromosome, then the number of linkage groups may equal but cannot exceed the haploid number of chromosomes. The mutants so far found in *D. obscura* fall into five groups, and there are five pairs of chromosomes.

Identical mutations

The present paper contains descriptions of 28 sex-linked characters which are due to mutant genes in 23 different loci. At least 6 of these show striking resemblances to sex-linked mutants in other species, and in the case of three the evidence is strong that they are the same. Even though only a small proportion of the mutants obtained seems to be identical, nevertheless, the results so far obtained tend to support the view that the identification of chromosome groups in different species may be possible through further work.

It was realized that the matter of determining homology by relying on similar mutants in different species to mark corresponding chromosome regions was a difficult one, and capable of leading to mistaken ideas, because quite differently located genes may, by mutation, produce indistinguishable somatic conditions; and similar character-effects may be produced by genes located in different chromosomes. For example, mutation of certain genes in the sex chromosome produces changes in either the eye color or wing shape very similar to the effects of mutations of certain genes in the autosomes. However, when two or more linked genes are found which stand in the same relative positions to each other in the two species and also produce the same somatic effects, the argument for regarding the genes as identical ones becomes very much stronger.

Chromosome rearrangement

The importance of the sequence of the genes may be shown by the following situation. Three closely linked genes in *D. obscura*, apparently identical with three in *melanogaster*, have been found on critical examination to have a different sequence in the two species. In *melanogaster* the order is yellow, white, notch; in *obscura* the order is yellow, notch, white. Had the sequence been the same in both cases, it would have seemed almost certain that the three characters were the same in each species. Then one must postulate some sort of a rearrangement, such as breaking off and re-attachment of a piece of chromosome. But it may seem more probable that one of the three is not the same character as the one it resembles in the other species. A more detailed consideration of this problem will be taken up later, after the various mutants have been described and their respective loci determined.

There are reasons for thinking that at least notch, white and eosin represent identical mutants, but there is a striking difference in their relative position in the two species in the chromosome map. In *melanogaster*, notch and white are closely linked and are both less than four units from the end of the chromosome map; in *obscura* they show a similar linkage value, but are at least 60 units from either end. These facts suggest that the size differences of the X chromosomes of the two species are due to the presence of a large piece of chromosome, attached at what corresponds to the left end of the *melanogaster* X.

DESCRIPTION AND LOCATION OF SEX-LINKED CHARACTERS

A multiple-allelomorphic locus and a modifying factor

Short (*s*)[2]

At the beginning of my work on *D. obscura*, Dr. C. W. Metz gave me a stock of a sex-linked recessive mutant named short. This mutant has been described by him (METZ 1916). It is characterized by a shortening of the fourth and fifth veins, which fail to reach the wing margin. The effect on the fourth vein is variable, however, and this vein is sometimes normal. In flies with wings that are not damaged, the character can be classified with precision by means of the condition of the fifth vein, and it has been much used in the various crosses. Its value is increased by the fact that the mutant flies are vigorous and of good viability, and that the nature of the somatic effect does not interfere with classifying any other sex-linked character that is used in the same experiment. When other sex-linked mutants were found which made it possible to construct a chromosome map, it turned out that short occupied a locus at the extreme right end of the group, and that about 170 percent of crossing over occurred between it and the other end.

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[2] In this section describing the mutants of the sex-linked group, the name of the character is followed by the symbol in parenthesis.

Shorter (s^r)

Shorter is a new mutant, allelomorphic to short, which appeared in culture 206, October 19, 1919. The mother was known to carry the gene for short in one chromosome (derived from her mother) and she should have received a normal allelomorph in the other chromosome from her father which was normal. However, she produced 40 sons which were short and 50 of more extreme type which were called "shorter." Later crosses of shorter males to short females produced daughters with the venation of the wings always affected. This showed that the two genes were allelomorphic. The gene for shorter occurred as a mutation from the normal gene. This mutant can be distinguished from short in that shorter affects all the longitudinal veins, second to fifth, and the gap by which they fail to reach the margin is greater than in short. The viability of shorter is good, and the character is infallible in its appearance and quite easy to classify.

Shortest (s^s)

The third mutant allelomorph in the short locus appeared in culture 329 as a single male, November 21, 1919. This was in the second count of the bottle, and no more "shortest" were obtained in the three subsequent counts. A total of 81 males were produced by the mother of this culture, which was homozygous for short. From this it is evident that shortest occurred as a mutation in the short gene in a maternal X chromosome either during meiosis or shortly before. In appearance, the shortest male is similar to the shorter male but more extreme. The longitudinal veins (second to fifth) of the wings are more shortened, and in some cases do not reach more than half way to the distal margin. The range of variation of shorter and shortest overlaps, so that they could not be separated from each other in all cases if used in the same experiment. In general, shortest males are distinctly more extreme than shorter males; so that in two homozygous populations the difference is easily discernible. This difference in range between the males of the two types does not hold for the females, which are quite similar in appearance. Their ranges of variation seem to coincide, although it is probable that biometrical methods would show the two curves to be not identical for the two types of females. The mutant shortest shows sexual dimorphism in that the males are more extreme than the females.

Dominance relations of the short allelomorphs

The normal gene is completely dominant to each of the three mutant allelomorphs in the short locus. By crossing short to shorter or to shortest, females were obtained which were the short-shorter or the short-shortest compounds.[2] In both cases the compound females looked like homozygous short females, and were not intermediate in condition. Short behaved as a dominant to both of its more extreme mutant allelomorphs. The shorter-shortest compound looked like homozygous shorter or shortest females, which have been already described as indistinguishable in appearance. Consequently, there is no question of dominance involved.

Gap (*g*), a disproportionate modifier of short

In the summer of 1919, Dr. METZ informed me that he had observed occasional flies in his cultures which had a gap in the fifth longitudinal vein, that is, the vein did not reach entirely to the margin of the wing. Such flies looked like extreme variants of short toward normal. Breeding tests showed that gap is inherited as an autosomal recessive belonging to the third linkage group, and in a homozygous condition acts as a modifier of short. Gap flies always have the fifth vein slightly shortened, and may or may not have a slight gap in the second vein.

Gap acts as a disproportionate modifier of short. BRIDGES (1919) has used this term to designate cases where the character shown by the double mutant type is different from what would be expected from the combined characters of the single mutant types. Flies which are homozygous for both gap and short have the second to fifth longitudinal veins of the wings shortened so that they do not extend to the distal margin. Such individuals are similar in appearance to shorter. Since the third vein is not affected by either gap or short alone, it was not anticipated that this vein would be distinctly shortened in the double recessive, and yet such is the case. For this reason, gap is considered a disproportionate modifier. It is a recessive modifier, since it acts only in the homozygous condition.

Gap and heterozygous short

Females which are heterozygous for short and homozygous for gap appear like ordinary homozygous short females, and were thought to be such when first observed. These short females were believed to be exceptions to the ordinary rule of sex-linked inheritance and to have occurred by non-disjunction of the X chromosomes of the mother. Breeding tests showed such females were only heterozygous for short, and that their appearance was due to the presence of gap in a homozygous state. This means that short, which is ordinarily recessive, does produce a distinct effect when heterozygous in the presence of homozygous gap. The latter condition makes short semi-dominant. A somewhat similar case in this respect was described by the author (LANCEFIELD 1918) for *D. melanogaster*, where a recessive mutation was semi-dominant in individuals homozygous for a specific modifier.

Gap and short-shorter or short-shortest compounds[3]

Gap does not make either shorter or shortest individuals more extreme, but it acts on the compounds of short with shorter or shortest just as it does on homozygous short. Owing to the dominance of short, these two compounds resemble short females, and are similar in appearance to each other. In other words, gap affects the compounds of short in the same way as it does short itself.

Beaded (*b*)

Beaded was found in culture 99, August 27, 1919. The culture produced six males which looked as though the margin of the wings had been regularly nicked. At intervals, the normal wing margin was left intact, and the dark bristles present in such areas formed isolated patches. The name "beaded" was applied to the mutant, but it is not the same as the autosomal dominant mutant of the same name in *D. melanogaster*. In culture 99, there were 72 females and 37 males produced, of which 6 males were beaded. This 2:1 ratio of females to males and the deficiency of beaded males can only mean that there was a sex-linked lethal in the same chromosome as beaded. The 6 males which appeared were crossovers between the lethal and the beaded genes. Three of these 6 were also crossovers with short, and were therefore double crossovers. The fact that 3 of the 6 beaded males carried a chromosome that had arisen by double crossing over indicated that beaded and short were far apart. It was impossible to say in which X of the mother the mutation occurred. From the ratio of sexes obtained and from the fact that the beaded males appeared in the second count and continued to appear to the end, it is evident that the mother of culture 99 was heterozygous for beaded and lethal. Her mother had produced a normal sex ratio. One other female from culture 13, out of 5 tested, had the same constitution as the one in culture 99, and this fact proves that the mutant genes beaded and lethal had been present in the parents. The data do not determine the stage at which the mutations occurred.

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[3] An individual which is heterozygous for two mutant allelomorphs in the same locus is called a compound.

Locus and valuation of beaded

Since beaded was the second mutant locus obtained in the X chromosome, it has been extensively used in later experiments. Its viability is nearly as good as normal, and both sexes are vigorous and breed readily. The character does not overlap normal and can always be classified unless the wings are badly damaged. The beaded effect is more pronounced in the males than in the females. Beaded does not interfere with the classification of any other sex-linked character, and only one other masks the beaded character.

From the first appearance of beaded, it was evident that its locus was far enough away from short to give frequent double crossing over. In 5 cultures, to test the linkage of short and beaded, 531 males were obtained, of which 249 or 46.9 percent were crossovers. However, since the region is long enough for double crossing over to occur freely, and since a double crossover could not be detected in a two-point cross, the region between must be much longer than 46 units. The determination of the locus of beaded in the chromosome had to wait until mutations were obtained involving intermediate loci. Suffice it to say here, that beaded and short were later found to be more than 140 units apart.

Fused (f_u)

Fused males were noticed in culture 565 on January 31, 1920, and, in all, 24 males of this mutant type and 3 normals were obtained. The mother was a female with a break in the fourth longitudinal vein of the wing. This condition was due to a dominant sex-linked gene which also acts as a recessive lethal and so kills the males of that genetic constitution. This mutant will be described later in this paper under the name of "broken." After the first fused males were observed, the culture produced 61 females and 27 males, which is in the ratio expected if half the males fail to survive. The great excess of fused males indicated that the gene for fused was in the opposite chromosome to broken (which is the lethal), and that there was about 12 percent of crossing over between the two loci. Since the mother in culture 565 received the X chromosome carrying broken from her mother, it follows that the X chromosome carrying fused was in the paternal one. The mutation to fused probably occurred in the germ track of the father. The exact stage cannot be decided.

Vermilion (*v*)

A vermilion-eyed male was found in culture 559 on January 30, 1920, and another on the tenth of February. The flies in the culture were taken out on February 7, and the second vermilion male hatched between that date and February 10. There was an interval of at least 8 days between the hatching of the two males. A single female was used as the mother of the culture. The number of males obtained from the bottle was not recorded, although 6 observations were made on the offspring to see if any stubby-bristle males would appear. The mutation to vermilion must have occurred in the mother of culture 559 at a late oögonial stage, as only a few gametes received the vermilion gene.

The color of the eyes of vermilion flies is lighter in shade and brighter than the normal color. The eye color of vermilion in *obscura* is strikingly similar to the autosomal eye color, scarlet, in the same species and in *D. simulans* and to sex-linked vermilion and autosomal scarlet in *D. melanogaster*, as well as to sex-linked vermilion in *D. virilis*.

Vermilion is a very valuable mutant and has been much used in placing other mutants in the chromosome. Eye-color mutants, in general, have good viability and, where the new color differs markedly from normal, are easy and certain of identification. Vermilion can be used in experiments with any other sex-linked mutants so far obtained except white. Vermilion was found to occupy a central position in the chromosome. Mutation has been especially frequent in that region. Vermilion, with short, beaded, and fused, was used in the first four-point experiment carried out, where the relative positions of these four loci were first determined. It early became evident that a four-point cross was necessary to determine whether vermilion was between beaded and short or at one end. The situation may be understood by considering the result obtained by crossing vermilion to beaded and short and obtaining the crossovers in F_2. Not anticipating any difficulty in detecting the double-crossover class, only three cultures were raised, and these produced 170 males. Since three loci are involved, there are two single-crossover classes and one double-crossover class, besides the non-crossover class, which may occur. The order, as determined by later crosses, is beaded-vermilion-short. Using this information to classify the kinds of males obtained, the 170 males were divided as follows: 39 non-crossovers, 34 crossovers in the first region, 60 in the second region, and 37 double crossovers. The three points were so far separated as to be nearly independent of each other.

Localization of genes in the chromosome

The relative position and distances of several loci were first determined with reasonable certainty by means of a four-point cross involving beaded, fused and short against vermilion. Four cultures produced 341 males which furnished information on the linkage relations. Only the males could be used, since the heterozygous females were not back-crossed to quadruple-recessive males, as these were not available at the time. The results of the cross are shown below. Inspection will show that one type of crossover class (beaded-vermilion-fused and short) is markedly smaller than the others.

Table 1

Nature of the cross: $\dfrac{\textit{beaded fused short}}{\textit{vermilion}}$

REGION	0		1		2		3		1,2		1,3		2,3		1,2,3		TOTAL
Class	*v*	*b* f_u *s*	*b* *v*	f_u *s*	*b*	*v* f_u *s*	*b* f_u	*v* *s*	*bv* f_u *s*	+	*b* *v* *s*	f_u	*b* *s*	*v* f_u	*b* *v* f_u	*s*	
	40	33	22	20	29	9	33	40	8	15	24	26	17	15	1	9	
Totals	73		42		38		73		23		50		32		10		341

On the basis of these data, the relative order of genes would be beaded, vermilion, fused and short in the order named. The observed amount of crossing over in this experiment is 36.4 percent between beaded and vermilion; 30.2 percent between vermilion and fused; and 48.4 percent between fused and short. A chromosome map constructed on the basis of these data, in which the loci are spaced according to these crossover values, is shown in figure 2. The observed crossover values in the three regions totals 115 percent of crossing over, which is more than had been obtained in any case so far recorded. It is evident to anyone familiar with the phenomena of crossing over in other species of Drosophila, that all three of the regions here involved are long enough to admit of considerable double crossing over; consequently, the distances indicated in figure 2 will be lengthened by the use of intermediate loci.

The order of genes adopted in figure 2 based on the 341 males recorded above has been completely verified by many later crosses made up in other ways and involving many additional genes.

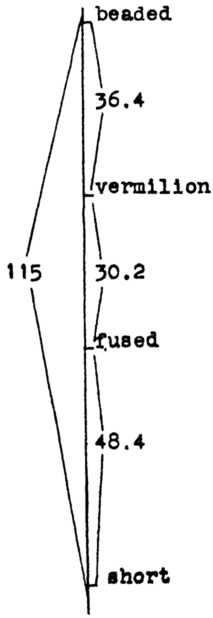

FIGURE 2.—Chromosome map based on cross involving beaded, vermilion, fused and short.

The frequency of triple crossing over obtained here is large. It is probably due to the length of the chromosome and the long regions involved.

Reduced (*r*)

Flies of both sexes which lacked the two anterior dorso-central bristles were first noticed in culture 465 on January 10, 1920. The name "reduced" was given to the mutant to distinguish it from an already existing mutant "minus" (not yet recorded in print) which looks like reduced, but is autosomal. Reduced behaves as a sex-linked recessive. Since reduced females, as well as reduced males, were obtained in culture 465, the father must have been reduced and the mother heterozygous for the gene, and this is quite possible as they were brother and sister. They doubtless received the gene in the X chromosome from their mother, culture 339. However, since the occurrence of flies with missing bristles escaped observation in culture 339, no conclusion as to the precise origin of the mutation can be drawn.

Reduced flies differ from normal, typically, in the absence of the two anterior dorso-central bristles. Occasionally only one of these is gone, and more rarely a posterior one is absent. Since the stock has been homozygous, no fly with all four dorso-centrals present has been seen, although several hundred individuals have been examined. Reduced has been used in many crosses because its locus lies between vermilion and fused in a region where numerous mutant genes have been discovered. Its classification is accurate, though not easy, owing to the necessity of carefully examining the number of bristles. It can be used in crosses without interfering with the classification of any other sex-linked characters. Its viability is slightly below normal, and it hatches more slowly. The exact locus of reduced was determined in experiments involving rough. These crosses will be discussed after a description of the latter mutant.

Rough (r_o)

Rough was found as a single male in culture 605, January 19, 1920. The culture had been counted three times, and the rough-eyed male was observed in the third count. A total of 60 males was produced. These facts indicate that the mutation to rough occurred in the germ track of the mother of culture 605, and that only a few gametes (possibly only one) received the mutant gene. The roughened appearance of the eye seems to be due to the fact that the hairs among the ommatidia are longer and become more prominent than normal, so that the surface does not appear as even as in the normal fly. Rough has proved to be a dependable mutant to work with, and can be used with most other sex-linked mutants. Glazed and compressed interfere with the classification of rough, as they produce great changes in the texture of the eye. Rough is inherited as a sex-linked recessive. Its viability is good.

Loci of reduced and rough

Reduced and rough have been located in the linkage group by crosses involving vermilion and fused. A cross of reduced rough to vermilion was made, and five cultures produced 376 males which were used to determine the order and crossover values. Since it took a double crossover to produce a vermilion reduced or a rough chromosome (the smallest classes), it is certain that reduced is between the other two in the chromosome. A further cross was necessary to determine whether reduced and rough were on the beaded or the fused side of vermilion. Accordingly, a cross of reduced rough against vermilion fused was made. In 253 males obtained, 3 were crossovers between rough and fused, which indicates rather close linkage between these two loci. The number and nature of the crossovers obtained fitted best with a sequence of vermilion, reduced, rough, fused, and considerations from later crosses of different nature bear this out. Reduced and rough have crossed over 74 times in 742 individuals and thus show 10 percent of crossing over. By adding 1.2 percent (value for rough and fused) a total of less than 20 is obtained for the vermilion fused distance, which is too low as compared with the value previously obtained.

TABLE 2

Nature of the cross: $\dfrac{\textit{vermilion}}{\textit{reduced rough}}$

REGION....................	0		1		2		1,2		TOTAL
Class..................	v	$r\ r_o$	$v\ r\ r_o$	+	$v\ r_o$	r	$v\ r$	r_o	
Total.................	315		23		30		8		376

Notch (*N*)

A female appeared in culture 455, December 26, 1919, which was called "notch" because of its similarity in several features to the notch mutants of *D. melanogaster* and *D. funebris.* This female was mated to wild-type males. Only three offspring were obtained: a normal female, a notch female, and a normal male. However, this was sufficient to show that the notch gene behaved as a dominant, as it does in the two other species mentioned. Fortunately, the one notch daughter bred better than her mother, and, crossed to beaded short males in culture 602, produced 46 notch females, 48 normal females, and 40 males all normal. Later crosses involving notch females always produced similar results: presence of notch and not-notch females and only not-notch males, and in general, a ratio of females to males of 2:1. Notch is inherited as a sex-linked dominant in its somatic effect and also as a recessive lethal. The notch females are always heterozygous and the normal allelomorph allows them to survive. However, since males have only one X chromosome, all males receiving a sex-linked lethal die, since no normal allelomorph can be present. This lethal action of notch operates to eliminate half the males and gives approximately a 2:1 sex ratio. The viability of notch females is poorer than that of normal ones, so that unless notch is run opposite to another mutant of poor viability, the notch females fall behind numerically, and there are not quite twice as many females as males. The notch mutant is named from one of several somatic peculiarities, namely, the posterior notching of the wings, particularly along the inside margin. A more reliable guide for identification consists in the thickening of the veins of the wings, especially noticeable at the juncture of the posterior cross vein and the longitudinal veins. The eight rows of microchaetae, or acrostichal hairs between the dorso-central bristles are disarranged and do not form definite rows as in the normal fly. The eyes may be somewhat roughened, though this can be made out in less than half the flies. The macrochaetae, in any part of the body, are frequently doubled, and on the scutellum one or more are often absent.

Other occurrences of notch

Notch$_2$ (N_2)

Notch mutations in *obscura* have occurred in three independent cases. The second occurrence of the notch mutation was discovered January 16, 1920, in culture 474, when two notch females were found. These two females, and also their progeny, showed the several somatic peculiarities of the original notch strain which was now designated as notch$_1$ to distinguish it from the second strain now established and known as notch$_2$. Notch$_2$ was undoubtedly a remutation of notch. The parents of culture 474 were a shorter female of shorter stock and a normal male of the Berkeley wild stock. The culture produced 105 daughters and 100 sons, which is sufficiently close to numerical equality of the sexes to show that the mother was not genetically notch. The notch females were found in culture 474 in the fourth or next to the last count of the bottle. They could not possibly have been due to contamination, as notch$_1$ was at this time being used for the first cross. The latter culture did not contain shorter, whereas both the notch$_2$ females from 474 were found to be heterozygous for it. If the notch mutation had occurred in a maternal X, the gene for shorter would be in the same chromosome as notch and if it had occurred in the paternal X, in the opposite chromosome. The progenies produced by these two females are recorded in table 3. It will be observed that in both cultures the number of shorter males slightly exceeds the number of normal males. One of these classes is a crossover class between notch and shorter. It is known from later experiments, involving intermediate loci, that the total amount of crossing over (as obtained by summation between notch and shorter) amounts to at least 90 percent. However, even in such a long distance the relatively greater frequency of double crossing over reduces the observed percent of separation, in most instances, to below 50. It is probable then that the 72 normal males represent the crossover class. From this it follows that notch and shorter were probably in the opposite chromosomes, and since shorter came from the mother, then the mutation to notch occurred in the father, and at least two sperm carried the notch gene. Ordinarily, the notch gene is never transmitted by the sperm, because males which are genetically notch are not obtained. Only when the mutatiòn itself takes place in the germ track of the male can the gene come from him. Notch$_2$ has been extensively tested genetically and behaves as a sex-linked dominant for its somatic effects and also as a recessive lethal.

TABLE 3

CULTURE	N_2 ♀	WILD-TYPE ♀	SHORTER ♂	WILD-TYPE ♂
604	73	99	51	46
603	36	52	29	26
Total..........	109	151	80	72

Notch$_3$ (N_3)

Notch$_3$ is known only from a single female which was found in culture 1101, June 12, 1920. The female showed the several peculiarities already described for notch$_1$ including the effect on the wings, veins, and bristles. She died without progeny. The occurrence of notch in this culture could not be due to contamination, since the notch$_3$ female was yellow as well as notch, and half her brothers and sisters were expected to be yellow. On the other hand, there were no yellow notch flies which could have contaminated the culture, as the yellow notch combination had not yet been obtained from other sources.

Locus of notch

Only notch$_2$ has been accurately placed in the linkage group, as notch$_3$ did not breed and the notch$_1$ strain died out before the proper stock was available to use in a three-point cross with yellow and white. In the crosses to yellow and white made individually, notch$_1$ showed close linkage to both. Notch$_2$ was crossed to reduced rough and the data derived from three cultures are summarized in table 4. Notch was shown to be to the left of both reduced and rough, since the rough males were clearly double crossovers. Since the amount of crossing over between notch and reduced is decidely more than that obtained between vermilion and reduced, the notch gene is assigned to a locus to the left of vermilion, or between vermilion and beaded. This location of notch to the left of vermilion agrees with the results of later experiments. The locus of notch was more definitely determined by a cross to yellow vermilion. Yellow is a recessive mutation affecting the body color, and will be described in a succeeding section of this paper. The summary of this cross appears in table 5. The absence of a double-crossover class leaves no doubt that the notch locus is situated between those for yellow and vermilion, and yellow is assigned to a locus to the left of notch, that is, between notch and beaded. As work progresses, it becomes evident that yellow and notch are not situated in close proximity to the left end of the chromosome map as are the yellow and notch of *melanogaster*. The more accurate placing of notch will be made after describing the yellow body color and the white and eosin eye-color mutants.

TABLE 4

Nature of the cross: $\dfrac{\textit{notch}}{\textit{reduced rough}}$

Females	*r* r_o ♂	+ ♂	*r* ♂	r_o ♂	Total ♂
248	71	29	11	2	113

TABLE 5

Nature of the cross: $\dfrac{\textit{notch}}{\textit{yellow vermilion}}$

REGION	0	1	2	TOTAL
Class	*y v* ♂	*v* ♂	*y* ♂	
Total	162	15	11	188

Deficiency nature of notch mutations

The nature of the change involved in the notch mutation in *Drosophila melanogaster* was studied by MOHR, who reported some interesting conclusions (MOHR 1919). It will suffice here to state that notch acts in some respects as a deficiency of an entire region and that the extent of the region affected varies in the different notch mutations of *melanogaster*. In the case of notch$_8$, the region extended as far as the white locus and acted as a deficiency for white. The phenomenon of deficiency was earlier discussed by BRIDGES (1917). In *D. obscura*, neither notch$_1$ nor notch$_2$ acts as a deficiency for white or its allelomorph, eosin. The color of the eyes of notch females which are heterozygous for white does not differ from the normal color. Notch$_2$ seemed to act as a deficiency for scutellar, a recessive mutation that removes some of the scutellar bristles. It was noticed that notch$_2$ females when heterozygous for scutellar had bristles missing on the scutellum more frequently than did notch females not carrying a gene for scutellar. However, notch$_2$ is not a deficiency for scutellar which is on the opposite side of yellow, and their combined behavior in removing scutellar bristles is probably better explained on the view that heterozygous scutellar strengthens the tendency, that notch mutations in *obscura* show, to remove some of the bristles.

Specific notch modifiers

In the case of *Drosophila melanogaster*, MORGAN (1919) has demonstrated the existence of genes which modify the character "notch." He found that these modifiers acted in different ways so that the notch condition was either increased or diminished according to the particular gene present. He was able to determine in which chromosomes the modifying genes were located.

In *D. obscura*, the condition of notch females in two instances has been strikingly changed due to the specific effect of modifying genes. Both would come in BRIDGES'S (1919 a) category of specific modifiers, since the genes produce no visible effect except in notch individuals.

Achaete modifier

In May, 1920, it was observed that some of the notch females in two inbred sister cultures, 1021 and 1025, were almost denuded of bristles. The parents of these two cultures were notch$_1$ females from culture 958 mated to brothers, and neither of the notch mothers was observed to differ from the typical condition of notch. Had they been achaete, as were some of their daughters, the condition would almost certainly have been noticed. The achaete individuals were always notch, although 258 offspring were produced and the not-notch out-numbered the notch by 2 to 1. The achaete notch$_1$ females were difficult to breed and the location of the modifying gene was not determined. The gene was shown to be recessive by out-crossing one achaete notch female. None of the notch daughters were achaete.

Split·modifier of notch

A second notch modifier was observed in the case of notch$_2$, November 18, 1920, in culture 1481 when a notch$_2$ female appeared with the wings considerably shortened and split about half way in from the distal end. There is a separation along this split so that a crude Y shape is assumed by the wing, that is quite conspicuous. The only flies which are affected are the notch females. Split notch$_2$ females are markedly below the wild type in vigor and productivity, but one mating (1577) was successful in which such a female was out-crossed to a minute-bristle male. Minute is an autosomal dominant mutant. Some of the split notch offspring were also minute-bristle which showed that the modifier was a dominant. No fly showed the split-wing condition which was not also notch. Split must then be considered as a specific modifier. This was again demonstrated by using a minute-bristle son from this culture, which also carried the modifier, to cross to a notch female from a stock that was known to be without the modifying factor. The offspring from this mating (1602) were 49 notch females, 47 not-notch females, and 12 sons. Although the minute-bristle father used here had normal wings, he carried the gene for split, as 26 of his 49 notch daughters showed the split-wing condition. Although half of the 47 not-notch daughters and of the 12 sons carried the modifying factor, they did not manifest any indication of it. The behavior of minute and split in this culture showed that they were carried in the same pair of chromosomes, and split is, therefore, a member of the third group of characters. This conclusion is based on the observation that none of the 26 split notch females were minute-bristle, while all the 23 unmodified notch females were also minute.

Yellow (*y*)

Mutation to yellow has occurred twice; first on March 24, 1920, when a few yellow males were obtained in the slender-bristle stock. The body, bristles, and wings are yellow in color and differ very strikingly from their dark gray color in the wild form.

Yellow$_2$ does not differ in appearance from the first yellow mutant. It arose through independent mutation from normal. Two yellow males appeared in culture 1040 in the third count, May 30, 1920, and two more in the fifth count, June 3. None appeared in two subsequent counts. Two recessive autosomal characters, scarlet and bithorax, were present in culture 1040. One yellow male was both scarlet and bithorax, while a second yellow male was bithorax. This is clear proof of the independent origin of yellow$_2$. Four yellow males were obtained in culture 1040 among 178 offspring, and these four came during the middle of the count. The mutation probably occurred in a maternal X chromosome not many divisions prior to maturation. Both yellows were inherited as sex-linked recessives, and by crossing were shown to be allelomorphs. The determination of the locus of yellow to the left of notch and vermilion has already been discussed. Yellow is a valuable mutant because of the ease and accuracy of its classification, its possible identity with similar mutants in other species, and because it can be used with any other sex-linked mutant. Its viability is fair. Yellow in *obscura* does not occupy the extreme end position in the chromosome map, but in order to make comparison easier, it is treated as the zero point, and map distance based on linkage is computed in both directions.

White (w_1)

The first mutation to white occurred in culture 641, when a white-eyed male was obtained February 13, 1920, in the second count of the bottle. Since no other white individuals were obtained in later counts, the mutation of a wild-type gene to white must have taken place in one of the X chromosomes of the mother at a late stage. The most conspicuous effect of the gene for white is the color of the compound eye and of the three ocelli. The white eyes have a grayish tinge, due probably to the hairs between the ommatidia, and are more similar in appearance to white flies in *D. melanogaster* that have an ebony body color than to the ordinary *melanogaster* white. The striking coloration of the abdomen in males of *D. obscura*, due to the orange color of the testes, is absent in white males. There is no such effect in the females, as the ovaries do not color the abdomen. White females have not produced offspring when mated to white or to any other males.

White$_2$ (w_2)

The second mutation to white took place in the reduced stock 592/2 where a few white males were found March 2, 1920. These white$_2$ males were similar in appearance to white$_1$, and the two were demonstrated to be allelomorphs when a white$_1$ male was mated to a female heterozygous for white$_2$, and white daughters were obtained. The independent origin of white$_2$ was demonstrated by the fact that it arose in the reduced stock, and the white$_2$ males were also reduced. There was no white in the pedigree of the reduced stock, and had these white males arisen by a contamination, they would not have been reduced.

White is a valuable mutant because of its possible identity with white in *D. melanogaster* which was strengthened by the finding of eosin, an allelomorph to white. The fact that white showed very close linkage to both yellow and notch added interest. It will be recalled that white eye in *melanogaster*, found by MORGAN in 1910, was one of the first Drosophila mutants discovered. White in *D. obscura* has poor viability and hatches later than normal. The females are sterile. For genetic use, white is not as valuable as eosin, as the presence of eosin does not prevent the classification of vermilion; and further the eosin females are fertile. Since white shows only one percent of crossing over with notch, the two mutants are kept in the same stock by mating white males to notch females, heterozygous for white. Since the white females present do not produce offspring even if they do mate with the white males present, the stock is automatically balanced for notch and white. The stock is occasionally examined to make sure that wild-type males have not arisen by crossing over.

Eosin (w_e)

Males with eosin-colored eyes were obtained in culture 1310, August 12, 1920, and were called eosin on account of their similarity to eosin in *melanogaster*. The *obscura* eosin have a darker tinge to the eye, as is also true for white. The mother of culture 1310 was a notch$_1$ female and the gene for eosin must have been in the opposite chromosome from that carrying notch. Judging by the count obtained, 104 females, 9 eosin and 1 wild-type male, a sex-linked lethal other than notch was also present. In order to get a sex ratio of 10 females to 1 male, the other lethal should give about 20 percent of crossing over with notch, as only half the crossovers will be free from a lethal. The exact location of the lethal was not determined. As the notch$_1$ female was heterozygous for eosin opposite notch, it is evident that the mutation to eosin occurred in the paternal X chromosome.

Eosin is intermediate in shade between white and wild-type. It is a dull reddish orange color. The color of the eye differs in the two sexes, being markedly darker in the females than in the males. This sexual dimorphism agrees with that in *D. melanogaster*, and strengthens the probability of the two being identical mutants. The ocelli also have the eosin color. The male abdomen is not colored by the testes.

Eosin is also a valuable mutant because of its close linkage to yellow and notch. It has much better viability than white and is more generally useful. It behaves as a sex-linked recessive and its classification is easy and certain.

White and eosin, with their wild-type allelomorph, form a multiple-allelomorphic series. White and eosin were shown to be allelomorphs when the white-eosin compound was obtained. Such females are intermediate in eye color and are a shade lighter than eosin males, which are lighter than eosin females. A similar relation holds for white and eosin in *melanogaster*.

The location of yellow, notch, and white or eosin

Preliminary crosses involving two loci at the same time indicated that yellow, notch, and white-eosin were closely linked. This was of interest because the three similar mutants in *D. melanogaster* are also closely linked. However, the sequence, as determined by a three-point cross, proved to be different. The cross was made up using eosin against yellow notch$_2$. A summary of the results is given in table 6. Inspection of the crossovers obtained shows the complete absence of representatives of a double-crossover class. The missing class would be yellow or notch eosin. The sequence is yellow notch eosin, which does not agree with the sequence in *melanogaster*, namely, yellow eosin notch. The result was a surprise, and while the above data seemed convincing enough, the cross was repeated in another way, using yellow eosin in one chromosome

TABLE 6

Nature of the cross: $\frac{\textit{yellow notch}_2}{\cdot}$

	FEMALE							MALE		
Region......		0		1		2		0	1	2
Class.......	Not classified	$y\ N_2$	w^e	$y\ w^e$	N_2	$y\ N_2\ w^e$	+	w^e	$y\ w^e$	+
	321	201	167	7	11	1	4	274	12	5
Total......	321	368		18		5		274	12	5

In this summary, the 321 females, not separately classified, occurred in cultures when a wild-type male was used. Only the males of such cultures could be used for crossover determination.

against notch$_2$ in the other. The results obtained in the second cross confirmed those obtained in the first. Four cultures are summarized in table 7. Here the class yellow or notch eosin, which was missing in the previous cross, is now present, while the triple-mutant and the wild-type class are absent. Both crosses indicate the same sequence, namely, yellow notch$_2$ white (or eosin). A consideration of the difference in sequence as compared with that of the similarly named mutants in *D. melanogaster* will be found in a later section of this paper.

TABLE 7

Nature of the cross: $\frac{\textit{yellow eosin}}{\textit{Notch}}$ ♀ × *yellow eosin* ♂.

	FEMALE						MALE			
Region......	0		1		2		0	1	2	1,2
Class.......	*N*	*y* w^e	*y N*	w^e	*y*	*N* w^e	*y* w^e	w^e	*y*	+
	214	175	10	9	5	1	152	14	1	0
Total.......	389		19		6		152	14	1	0

Having determined the sequence, the map distances may be calculated, using all the available data for these two regions, which is given in the regional summary. A yellow notch distance of 5 units is obtained, and a notch white (or eosin) distance of 1 unit. These three loci have their position in the linkage group to the left of vermilion. The yellow vermilion distance is about 14 units, with notch and white between. The sequence of the genes in the chromosome map is now beaded, yellow, notch$_2$, white (or eosin), vermilion, reduced, rough, fused, and short.

Scutellar (s_c)

The absence of some of the scutellar bristles on some males in slender-bristle stock was observed April 26, 1920. The character scutellar was somewhat variable as regards the absent bristles. Commonly, the two posterior bristles of the scutellum were missing, but occasionally they were present, while one or both of the anterior scutellar bristles were absent; and less frequently all four were gone. The post-vertical and ocellar bristles of the head, which are removed in the *melanogaster* scute, are not absent in this case. Judging from the observations made on the homozygous stock of scutellar, the character always shows itself. Scutellar is inherited as a sex-linked recessive.

Locus of scutellar

The gene for scutellar was located in the chromosome mainly by crosses involving yellow. The linkage between these two loci was very strong, as only four crossovers were obtained in a total of 1268 individuals. This gives 0.3 percent of crossing over. In order to determine whether scutellar is located to the right or left of yellow, a cross was made using yellow scutellar in one chromosome against eosin in the other. A summary of the result follows. Only the males were used for linkage. Evidently, scutellar eosin and yellow are single crossovers between scutellar and yellow. The two scutellar eosin flies constitute the critical crossovers. Both were tested by mating them individually to two virgin scutellar females to see whether they were genetically scutellar. In both cases, their scutellar constitution was demonstrated by the production of daughters which were uniformly scutellar. There is, thus, no doubt that the scutellar locus is to the left of yellow, that is, on the side away from notch.

TABLE 8

Nature of the cross: $\frac{\textit{scutellar yellow}}{}$

Region............	0		1		2	
Class............	$sc\ y$	w^e	$sc\ w^e$	y	+	$sc\ y\ w^e$
	166	141	2	0	12	3
Total............	307		2		15	

Scutellar is a good workable mutant, but is not used very extensively, as its locus is so close to yellow which is more easily and accurately classified. Bristles may occasionally be missing from the scutellum in flies which are not genetically scutellar. Both sexes are vigorous and productive and the viability is good.

Pointed (*P*)

A female with long narrow wings, pointed distally, was observed in the third count of culture 482, January 10, 1920. This was the only individual of the kind obtained in the culture which produced 152 flies and was counted five times. In places where the wing is reduced, the heavy margin is absent as though it had been trimmed away. The typical condition of pointed-winged flies is very similar to the picture of beaded in ***melanogaster***, which is published in "The physical basis of heredity" by MORGAN

(1919) opposite page 258, although there is probably no genetic identity between the two. Since only one pointed fly was found in 152 offspring (although pointed is inherited as a dominant and would show itself in individuals which received the gene), it is reasonable to conclude that the mutation occurred at a very late stage, either during or shortly before maturation. There is no way of determining in which parent the mutation occurred.

Pointed is a dominant which is unfailing in its appearance, and for that reason useful. Its locus was found to be to the left of beaded, at the end of the chromosome map, to which it added about 20 units of length. Its classification is rapid and accurate. One thing mars its usefulness, namely, the fact that the pointed condition makes it impossible to classify for beaded, which is so located that it is frequently desirable to use them together. Since the reduction of the wings affects the venation, the wings are easily crumpled. It is difficult to classify for short or fused in pointed flies. Both sexes have excellent viability and good productivity.

Locus of pointed

Pointed was assigned an approximate locus in the chromosome map to the left of beaded, or on the side away from yellow. A critical cross of pointed to beaded vermilion was made after pointed had been crossed to various sex-linked mutants and had shown apparently free crossing over to all. Pointed and beaded were not crossed until as a last resort for the reason that the presence of the pointed condition made it impossible to determine whether an individual was also beaded or not. However, the linkage of the two mutants could be estimated in the cross of pointed against beaded by doubling the normal class, since, as stated, its reciprocal crossover class, pointed beaded, could not be distinguished from pointed. As the viability of the normal individuals is higher than that of the beaded, the result obtained by doubling the normals will be somewhat too high.

The males in three cultures of the cross of pointed against beaded vermilion were classified as in table 9. Of the two classes, wild-type and vermilion, which contain neither pointed nor beaded, one is a single- and the other a double-crossover class. The discrepancy in size of the two immediately suggests that the wild-type is the single-crossover class. Now, the reciprocal class of normal, or the pointed beaded vermilion flies, were counted among the pointed vermilion, and in the same way the pointed beaded were not separated from the pointed. It is clear that the pointed vermilion class outnumbers its reciprocal class more than does the pointed. If this is because the pointed beaded vermilion exceeded the pointed beaded in numbers, it would confirm the view that the double-crossover class is represented by vermilion. By doubling the crossovers enumerated in the normal and vermilion classes, the percent of pointed-beaded crossing over obtained was 27.4.

TABLE 9

Nature of the cross: $\frac{\textit{pointed}}{\textit{beaded vermilion}}$

Class	*P* (*P b*)	*b v*	*P v* (*P b v*)	*b*	+	*v*	TOTAL
Total	52	40	52	32	21	7	204

Glazed (g_z)

The first count of culture 1163, July 2, 1920, showed the presence of two males with a new eye condition. The surface of the eyes was smoothed over, and had lost the slightly spiny condition of the normal eye. From the fact that only two males appeared and both in the first count, the origin of glazed may be traced to a mutation previous to maturation in one of the egg strings of the mother of culture 1163. Since one glazed male was vermilion and the other was not, there is no way to determine whether the mutation occurred in the maternal or paternal X chromosome.

Glazed is inherited as a sex-linked recessive. The viability of this mutant is a trifle below normal. The homozygous females have never produced any offspring even when used in mass cultures. The stock is kept going by mating glazed males to heterozygous females. Since the locus of glazed is close to that of pointed, it is fortunate that glazed can be used in crosses with either pointed or beaded without difficulty of classification. Glazed can also be used with any of the various eye colors. Aside from the sterility of the females, glazed has some distinct advantages over pointed, as it can be more generally used.

Locus of glazed

One of the original glazed males was yellow vermilion short, as well as glazed, and this quadruple recessive male was crossed to a wild-type female of Berkeley stock. From the results obtained from five cultures in this four-point cross, the locus of glazed seemed to be to the left of yellow. The other original glazed male was also yellow and short. It was mated to a fused female and three cultures were counted for linkage relations. The results here indicated the same location as had the preceding cross. It was evident that glazed was in the pointed beaded region.

Cross of glazed by beaded

A cross of glazed by beaded vermilion showed that the location of glazed, based on the two crosses already mentioned, was correct. This latter cross made it possible to determine the locus of glazed to the left of beaded, that is, on the same side as pointed. A glance at the summary of the two cultures will show that while 21 crossovers were obtained between beaded and vermilion, and 14 between glazed and beaded, there were no simultaneous crossovers in both regions. The sequence is glazed beaded vermilion. In crosses involving glazed and beaded 39 crossovers have occurred among 242 individuals, which gives a percentage of crossing over equal to 16.1.

TABLE 10

Nature of the cross: $\frac{\textit{glazed}}{\textit{beaded vermilion}}$

Region.........	0		1		2	
Class..........	$b\ v$	g_z	$g_z\ b\ v$	+	$g_z\ v$	b
	25	18	9	5	11	10
Total..........	43		14		21	

Cross of glazed to pointed

The previous crosses in which glazed has been used would indicate that its locus is not far from that of pointed, and a cross was made to get data on this point. Among 176 individuals, there were 7 crossovers, which gives 4 percent for a crossover value. Since the glazed beaded value plus the glazed pointed value together equal 20 (which is less than the estimated pointed beaded distance) glazed is tentatively assigned to a position between pointed and beaded. This is now in process of being tested by a cross involving three loci, simultaneously.

Broken (B_r)

During the third count on culture 418, two females were discovered December 18, 1919, which had a region of the fourth longitudinal veins missing, or much weakened. Later it was observed that the eyes of broken flies may be somewhat rough. The character is inherited as a sex-linked dominant in the females, but no broken male survives. Cultures raised from broken females show the same lethal ratio as has been described for notch. The gene for broken may be assumed to act as a dominant for its somatic effect and also as a sex-linked recessive lethal. The origin of the mutant gene took place at a late stage in the germ track of one parent, whether of the father or the mother is uncertain.

Broken is not a valuable mutant, as the females do not always show their genetic constitution and were difficult to breed. The stock can be kept going only by using broken females.

Locus of broken

A cross of broken to vermilion rough showed that the locus of broken was intermediate between those two (see table 11). If broken is between vermilion and rough, it would take a double crossover to produce a wild-type male and none were obtained. Broken has not been used in a three-point cross with reduced, and consequently, the relative position of these two genes is uncertain, although they are undoubtedly very close together. Based on the comparative linkage to vermilion, reduced is closer to vermilion than is broken. Between vermilion and broken, 55 crossovers occurred among 508 individuals, or 10.8 percent of crossing over took place. For vermilion reduced, a crossover value of 8.4 percent is obtained, based on 668 individuals. On these data, which are not conclusive, broken is assigned to a locus between reduced and rough.

TABLE 11

Nature of the cross: $\frac{\textit{broken}}{\textit{vermilion rough}}$

Class.	$v\ r_o$	v	r_o	+
Total .	77	7	5	0

Slender (s_l)

The character known as slender was discovered in culture 651, February 11, 1920. The mother used in the culture was evidently heterozygous for slender, as 17 of the sons were slender and 17 were not. Slender is inherited as a sex-linked recessive. The mutant was named from the change produced in the bristles, which are longer and smaller and more cylindrical than normal. They do not taper as the normal ones do.

Slender has not been used very much because it is located close to short which is somewhat easier to classify. Slender can always be distinguished from normal, and can be used with nearly all the other sex-linked mutants.

However, its classification is not rapid, as a careful examination is necessary in each case. The viability is good and both sexes are productive.

Location of slender

Slender was early crossed to short and found to give about 8 percent of crossing over with it. It was not determined which of the two occupied the end locus until crosses were made involving ascute and also compressed. These experiments showed that the sequence is compressed, ascute, slender, short. Ascute and short had been found to give about 22 percent of crossing over. This is approximately equal to the sum of the values obtained for ascute slender plus slender short. A four-point cross gave data confirming the location of slender between ascute and short.

Compressed (c_o)

In the first count, March 15, 1920, culture 803 produced four males of a mutant type which was called compressed. Such males were obtained throughout the time the culture was under observation and, in all, 36 compressed to 54 normal males were produced. The mother of culture 803 was heterozygous for beaded and short (in opposite chromosomes) as well as for the new mutant gene, compressed, but the compressed crossed over so freely with both that it could not be determined whether compressed was in the same chromosome with beaded or with short.

The mutant was named "compressed" because one of its most noticeable effects is a decided antero-posterior shortening of the thorax, so that the anterior dorso-central bristles, which are sometimes missing in this mutant, are back very close to the posterior ones. There frequently occurs a mid-dorsal depression on the thorax which is marked anteriorly. The small bristles, or acrostichal hairs, on the thorax are disarranged and do not run in regular rows as in wild-type flies. The reduction in size of the eyes is variable. Only a few ommatidia may remain, or all may be gone. Compressed individuals vary exceedingly in the difference in the condition of the two eyes. Cases are common where the eye on one side is very small and on the other side is nearly wild-type in size.

The poor viability of compressed causes it to run considerably behind wild-type in numbers, and, consequently, detracts from its genetic usefulness. It is located close to fused, which is a better mutant for most purposes. Compressed has some interest as being another case where a gene produces a number of diverse effects. It is inherited as a recessive.

Locus of compressed

The results obtained in culture 803, where compressed first appeared, made it evident that the gene for compressed was not near either to beaded or to short. Later crosses show close linkage with fused, as only 5 percent of crossing over was observed among 703 individuals. It has not been determined on which side of fused compressed is located.

Ascute (a_s)

A male was obtained in culture 1302, August 11, 1920, which resembled the autosomal mutant ascute of *D. melanogaster*, and an undescribed autosomal mutant of *obscura* known as grooveless. The new mutant was named ascute but as it turned out to be sex-linked, it probably is not identical with the autosomal scute of *melanogaster*. My records do not show how many ascute males were produced by culture 1302, as they were at first suspected of being a mutant already known, "grooveless."

In normal flies, there is a distinct groove separating the thorax and the scutellum on the dorsal side. This groove is absent in ascute. Ascute flies frequently show what appears to be an excrescence of brownish substance on each side of the body at the juncture of the thorax and the scutellum.

Ascute is a recessive. Its gene lies about 22 units from short, between fused and short. It is important in that it fills in the long gap to the left of short. The character can be identified in all individuals of that constitution. It can be used with any other sex-linked mutant without either being masked by the presence of the other. The viability of ascute is not good, as it usually runs distinctly below equality with the wild-type. The individuals are lacking in vigor and are short-lived.

Location of ascute

The first cross using ascute also involved pointed and yellow, and did not yield a very definite answer as to the locus of ascute except that it was a long way from either pointed or yellow. In this cross, 245 males were observed and the double-crossover class of 51 exceeded one of the single-crossover classes by 4. A cross using ascute short in one chromosome, against compressed in the other, gave a more definite result. Assuming that the 41 class is the double-crossover one, the sequence is compressed ascute short, and the ascute short distance is about 22 units. The crossovers between compressed and ascute amount to 44 percent of the total. A large amount of double crossing over doubtless occurs in such a long region, but cannot be determined with accuracy until other mutants with intermediate loci are available to work with. In *D. melanogaster* a direct crossover value of 44 percent would indicate a map distance of approximately 60 units. However, it is not yet ascertained whether the observed separation frequency for long distances has the same relation to map distance in *D. obscura* as in *D. melanogaster.* Consequently, it is not entirely legitimate to correct for double crossing over in *obscura* with estimations based on the other species.

TABLE 12

Nature of the cross: $\frac{\textit{ascute short}}{\textit{compressed}}$

Region	0		1		2		1,2		
Class	c_o	a_s s	c_o a_s s	+	c_o s	a_s	c_o a_s	s	
	95	91	50	98	26	27	13	28	
Total	186		148		53		41		428

Miniature (m_n)

A single miniature-winged male appeared in culture 1253, July 27, 1920. The mutation took place in the mother probably at a late stage in the formation of her gametes. The wings are normal in shape but are reduced to about two-thirds of the normal size and slightly darkened. The entire individual is smaller but not to the same extent as are the wings. While miniature in *obscura* resembles miniature in *melanogaster*, the use of the same name is not intended to signify that they are identical mutations.

Miniature is a recessive mutant of good viability and can be identified without difficulty. Its locus is between vermilion and reduced, which limits its usefulness since vermilion is more generally useful.

Locus of miniature

Miniature was used in a cross with vermilion and fused. Out of 224 individuals, 4 crossovers occurred between vermilion and miniature, and, in 3 of the 4, fused did not stay with vermilion. Miniature is placed, therefore, between vermilion and fused at a distance of 1.8 units from the former and 28.6 units from the latter. That this is the location of miniature is made more probable from results in a cross of miniature to white reduced. The results of this cross may be found in the table of summarized experiments. The position of miniature is clearly shown to be about midway between white and reduced. Miniature and reduced have crossed over 8.6 percent of the time among 326 individuals. This amount of crossing over is less than that between vermilion and reduced. The experiment, which has just been referred to, shows in a striking fashion the poor viability of white. In every culture, each class containing white ran behind its reciprocal. More data are required before miniature can be accurately placed.

Singed (s_g)

A white male with abnormal bristles was found in culture 1312, October 15, 1920, during the second count of the bottle. This was the only male showing the new bristle characteristic, although three later observations were made on the culture, which produced, in all, 48 males. Since the new mutant, "singed," has shown only 6 percent of crossing over with white, singed probably arose by a mutation in the same X chromosome of the mother which carried the gene for white. The mother of 1412 was heterozygous for white and scutellar in opposite chromosomes. White had been received from her father. It is reasonable to conclude that the mutation to singed took place in the paternal X chromosome of the mother about the time of maturation, as it is certain that only a few gametes, at most, received the mutant gene, possibly only one.

Singed flies have the large bristles and the acrostichal hairs twisted and bent. The general effect is similar to that in the singed mutant of *D. melanogaster*. The females are sterile, as is the case with the more extreme allelomorph in the *melanogaster* singed. Singed is a recessive. Aside from the sterility of the females, which makes it impossible to keep a homozygous stock (entailing the necessity for selection in each generation), singed is a good mutant to work with. Its viability is fair. Its genetic usefulness is limited owing to its location close to vermilion. Whether singed is the same mutant as the similarly named mutant of *melanogaster*, or only a mimic cannot be determined from the present evidence.

Locus of singed

The approximate location of singed was determined by a cross of the original white singed male to a fused short female. The linkage relations were calculated from the males of six F_2 cultures (see table 17). The sequence which best fits the data, gives an order of white, singed, fused, short. The observed crossovers amount to 6 percent between white and singed, and 26 percent between singed and fused. These values are based on 456 individuals. As the two values combined give a crossover value of 32 for white and fused, which is in good agreement with the value for the same region estimated from other crosses, we may assume that the conclusions arrived at as to the locus of singed are reliable.

A three-point cross, using singed against yellow vermilion, was made to determine with certainty on which side of vermilion singed had its locus. The males from four F_2 cultures are summarized in the table below. The absence of a double-crossover class gives the order yellow vermilion singed. Seven crossovers in 327 individuals give singed a position 2.2 units away from vermilion and very close to the probable locus of miniature.

TABLE 13

Nature of the cross: $\frac{\textit{yellow vermilion}}{\textit{singed}}$

REGION	0		1		2		TOTAL
Class	$y\ v$	s_g	$y\ s_g$	v	$y\ v\ s_g$	+	
	147	133	15	22	4	3	
Total	280		37		7		324

Polished (p_o)

The mutant polished was discovered in culture 1181, July 7, 1920. The female used in the culture was heterozygous for polished, which must have been in the opposite chromosome from yellow. It came, therefore, from the X chromosome of the father.

The polished males differ exceedingly in their appearance according to whether or not they are also slender. By itself, the gene for polished makes the body darker and shiny, and makes the bristles shorter and heavier. However, polished and slender together produce an unexpected result, as the bristles are made longer and are twisted in various ways. Polished is a recessive mutant. It would be more useful if it were located elsewhere in the chromosome than close to vermilion. With experience, polished can be identified accurately, though not rapidly, and can be used with all the other sex-linked mutants. Both sexes are fertile and the viability is fair.

Locus of polished

As polished appeared in a culture already heterozygous for yellow, it was apparent almost immediately that these two mutants were located in the same region of the chromosome. Only two males out of 46 are recorded as crossovers, but it is probable that the classification at that time was not accurate, especially as yellow and polished are not a favorable combination of characters.

Polished was crossed to rough and showed a crossover value of 14 percent based on 140 flies. Bearing in mind that yellow and rough are about 34 units apart, the results then available indicated that polished was between yellow and rough, somewhere in the vicinity of vermilion. Polished was crossed to white reduced and shown to occupy a position between them. However, the exact position of the polished locus between vermilion and reduced must depend on data yet to be procured. The relative order of the three genes between vermilion and reduced, namely, singed, miniature and polished, is not yet ascertained with accuracy.

Dwarf (d_w)

Males, which were distinctly small in size, were observed in two sister cultures, 448 and 449, on December 23, 1919. These males were otherwise normal in structure. It was found that so much variation in size occurred that dwarf overlapped normal and made accurate classification impossible. For this reason the stock was not retained. The two mothers of cultures 448 and 449 were heterozygous for short as well as for dwarf, and in opposite chromosomes, as was shown by the fact that the dwarf flies were mostly not-short. The parents of the cultures that produced dwarf were taken from culture 291 where a short male was mated to a heterozygous short female. The mutation to dwarf must have occurred in the not-short X chromosome of that female and before maturation. The linkage of dwarf and short was determined roughly. Twenty-two crossovers were obtained in 218 males, or 10 percent of crossing over. Whether dwarf was to the right or left of short in the X chromosome was not determined.

Deformed (d_f)

Deformed was found in culture 257, November 3, 1919. The mother was apparently heterozygous for the gene, so that the mutation must have occurred in one of her parents. In deformed, the eyes do not have the regular curvature but are cone-shaped; the thorax is shortened; and the wings held out away from the body. The scutellum is flattened. Many of the vibrissae about the proboscis are absent. I am informed by Dr. A. H. STURTEVANT that this last condition is normal in certain other species of Diptera. Only one sex comb is present on the front legs, as the tarsal joint which bears the second one is absent. These are the chief bodily effects produced by the gene, but there are many other lesser departures from normal which it would be difficult to describe. The deformed males did not breed, probably because they lacked enough vigor to mate. They were short-lived and succumbed to lack of food or to dryness far more readily than did normal individuals.

The location of deformed was not determined, as deformed is not the type of mutant which is useful in breeding or linkage experiments.

Extended (e^d)

The mutant extended was found in culture 953, April 15, 1920. The mother of this culture carried notch$_2$ in one chromosome and reduced rough in the other. The new gene was in the chromosome opposite notch, and more distant from notch than either reduced or rough. The mother received this reduced rough chromosome from her father, and the mutation must have occurred in it. On the rather meager data furnished by this culture (41 males), extended was placed about 26 units to the right of rough.

The mutant was named from the position in which the wings are held. They are spread away from the body, and curved downwards toward the ends. The mutant was not a useful one, as the individuals were weak and the males did not breed. It could have been kept only by breeding from heterozygous females, and was not retained.

Stubby (s_b)

Males with short stubby bristles were found by Dr. C. W. METZ in his culture L 553, December 11, 1919. Their main characteristic is the shortening of the macrochaetae and a bending or twisting of the bristles that occurs rather infrequently. It does not resemble the forked mutants of other species. This mutant was placed at my disposal by Dr. METZ. A cross of stubby to short showed that the two were closely linked, as only two crossovers were obtained in 150 individuals. Unfortunately, the further location of the gene for stubby could not be carried out as the strain was lost.

Lethal factors

Numerous sex-linked lethal factors have arisen by mutation during the investigation. Two of these have been discussed by the author (LANCE-

FIELD 1919) with reference to the nature of the sex ratio which resulted. The presence of a sex-linked lethal becomes evident, as only half the expected number of males is obtained. Such factors have been located in various regions of the chromosome map but are not sufficiently significant to merit attention here.

CONSTRUCTION OF THE CHROMOSOME MAP

The evidence showing that genes are arranged in linear series need not be presented here, as it has been adequately treated by STURTEVANT (1914, 1919), MULLER (1916), MORGAN (1919 a), BRIDGES (1919), and elsewhere. The chromosome map is one way of expressing a composite view of the linkage relations of the genes. The accuracy of the map depends on the statistical significance of the data from which the map is constructed. A brief statement of the essential points involved in the procedure is given by MORGAN and BRIDGES (1919).

It should be recognized that the amount of crossing over between the genes is not an absolute measure of their distance in the chromosome. There is probably no change in the distance relations of the genes which corresponds to the variations of linkage obtained by age change (BRIDGES 1916), or by temperature variations (PLOUGH 1918), or through presence of certain genetic factors (STURTEVANT 1919). It has been shown, in fact, that such linkage variations do not alter the sequence of the genes. There are some indications that the same relationship between chromosome distance and percent of crossing over does not hold in all regions of the same chromosome.

In making the map for the X chromosome of *D. obscura*, the gene for yellow has been taken as the zero point (although it is not a terminal gene in the linkage group) because the genetic length of the region between yellow and beaded cannot be determined until some suitably situated intermediate mutants are discovered. The region between yellow and fused, however, is filled with many genes so spaced that it is possible to work out the map distances with a fair degree of accuracy. Yellow is also used as the zero point to facilitate comparison of the sex-linked group of mutants with the sex-linked groups of other species of Drosophila. It is, however, still doubtful as to whether yellow should be considered an identical mutation to the yellow of other species.

The previous section of this paper has presented the evidence upon which the sequence of the mutant genes was based, and has indicated the method by which the various regions were oriented with respect to the others.

The loci of beaded, yellow, vermilion, fused, and short were early determined, and have been used as points of reference for the placing of other genes in their relative positions. The yellow-beaded distance is given as 42 units, which is based on the direct linkage value not corrected for a large amount of double crossing over, which undoubtedly occurs. Another long gap is shown between fused and ascute, and this again is based on the direct crossover value. Each of these regions will be considerably lengthened by using intermediate loci so that double crossovers can be detected. It was not thought profitable to attempt to calculate a correction based on the coincidence obtained in *D. melanogaster* until more evidence is secured that the coincidence relations are similar in the two species.

There is some difficulty in deciding what the distance between vermilion and fused should be. The sum of the intermediate distances is several units less than the distance based on direct crossing over. The first experiment, using vermilion and fused with no intermediate points, gave 30 percent of crossing over. However, the values obtained for vermilion reduced, reduced rough, and rough fused are: 8.4, 10.0, 1.2, which total less than 20. The discrepancy may be due partly to the use of values based on small numbers, as in the case of the rough-fused distance. The data for vermilion singed and singed fused are more extensive and give a total vermilion-fused distance of 28.2. The distance of 28 is adopted, which puts vermilion at 14 and fused at 42. Reduced is placed at 24 and rough at 36 until more extensive data are forthcoming.

In several cases the sequence is still in doubt. Such cases are compressed and fused; and the three genes between vermilion and reduced, namely, miniature, singed and polished. The order of the genes has been determined in most cases by crosses involving three or four loci in the same experiment.

The completed chromosome map is 170 units long. Crossovers are so frequent that three loci located at the two ends and in the center of the chromosome, respectively, show free crossing over with each other. This great length of the X chromosome in comparison with that of *D. melanogaster* and *D. virilis*, both cytologically and genetically, seems to be due to the addition of a considerable piece of chromosome, the source of which is, as yet, undetermined. The results indicate that in a general way the amount of crossing over in a chromosome is roughly correlated with its cytological length.

MUTANTS SIMILAR TO THOSE OF OTHER SPECIES AND CORRESPONDING CHROMOSOME REGIONS

It is not yet possible to decide just what region of the X chromosome of *D. obscura* corresponds to the X chromosome of *D. melanogaster* or

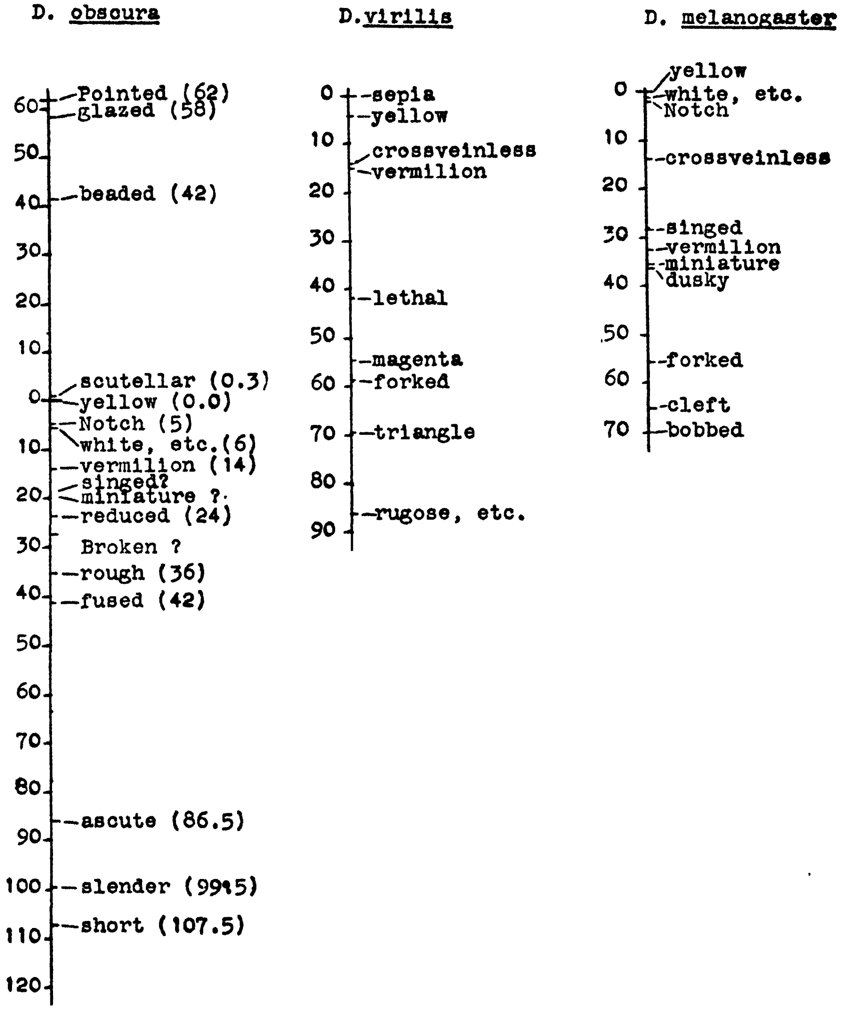

FIGURE 3.—Map of X chromosomes of 3 species of Drosophila.

D. virilis. Several sex-linked characters have been described which resemble in a striking manner those found in the sex-linked group of other species. However, the sequence, in the case of yellow, notch, white, is different in *melanogaster* and in *obscura*, and this difference makes any comparison uncertain.

The best method for determining that similar mutants in different species are identical, is to cross them and see whether they behave as allelomorphs. STURTEVANT (1921) crossed several sex-linked mutants of *D. melanogaster* and *D. simulans*, and showed that the mutant genes behaved as allelomorphs. Since interspecific crosses, using *obscura* and other species, have not been successful, this test cannot be applied here.

A brief comparison of mutants which resemble ones in other species may now be made.

Notch

Notch is a unique character in several respects and agrees very closely with the notch character in *D. melanogaster* and *D. funebris*. As STURTEVANT (1918) pointed out, when two mutants compared have several characteristics in common, the probability of identity is strengthened.

White and eosin

White and eosin are allelomorphs, as in *D. melanogaster*, and also show very close linkage to notch. The sexual dimorphism of eosin is similar in both species and the white-eosin compound is intermediate in eye color. A point of difference is the sterility of white females in *obscura*.

Yellow

This mutant resembles the yellow mutants of *virilis*, *melanogaster*, and *simulans*, of which the last two were shown to be actual allelomorphs by STURTEVANT (1921). The fact that the sequence of yellow, notch, and white eosin in *obscura* differs from the order of the similarly named characters in *melanogaster* makes the identity of yellow in *obscura* with the other yellows uncertain. The difference of sequence might be due to a rearrangement of that part of the chromosome, as BRIDGES (1917, 1919) suggested in his cases of duplication and transposition. If such a rearrangement has taken place, the characters may be identical with those of *melanogaster*, in spite of the change of sequence. At present, the case for identity seems to be weaker for yellow than for notch or white eosin.

Scutellar

Scutellar agrees with *melanogaster* scute in removing scutellar bristles and in showing very close linkage to yellow. In *melanogaster* no crossovers have been obtained, while in *obscura* 4 crossovers occurred among 1268 individuals. Scute has no post-vertical or ocellar bristles on the head. These bristles are present in the *obscura* mutant. The general similarity of scute and scutellar and their linkage to yellow in each case may be only coincidence.

Vermilion

Vermilion is very similar in appearance to sex-linked vermilion in *virilis* and *melanogaster*, and also to autosomal scarlet in *obscura*, *melanogaster*, and *simulans*. In such a situation, similarity of appearance does not give very good evidence for genetic identity. The crossover value for yellow and vermilion is about 14 units in *D. virilis*, according to information furnished me by Dr. METZ, which agrees with the value found in *obscura*. The fact that the double-recessive eosin vermilion is nearly white in color in *D. melanogaster* and lemon in *D. obscura* suggests that the vermilion gene in *obscura* is not the same as the vermilion of *melanogaster*. The eosin vermilion map distances are 8 and 31, respectively, in the two species.

Singed

Singed agrees fairly well as regards its several peculiarities (including sterile females and its map distance from yellow) with *melanogaster* singed. In general, little reliance can be placed in bristle characters of this type for determining identity of mutant genes unless corroborative evidence is furnished by other closely linked characters, since both in *D. melanogaster* and in *D. willistoni* there is more than one sex-linked locus which is capable of producing mutations of this type.

Miniature

This character was named on account of its resemblance to *melanogaster* miniature, and it is an interesting coincidence that miniature in both cases is located just to the right of vermilion in the linkage group. Since the two vermilions do not seem to be the same, the identity of the miniatures is very doubtful.

Glazed

Glazed resembles the *virilis* glazed in the way the eyes are affected; and wax, an allelomorph of *virilis* glazed, has sterile females as does *obscura* glazed. In order to regard glazed as the same locus in the two species, it would be necessary to disregard the yellow vermilion comparison, which would orient the chromosomes in the other direction.

Ascute

Ascute is not similar to any sex-linked characters in other species, but it agrees very well in its effects with *melanogaster* ascute, with *obscura* grooveless, and with *virilis* hunch, all three of which are autosomal. There is no groove between thorax and scutellum, and a bit of brownish substance which looks like an excrescence is frequently at the junction of the thorax and scutellum. Ascute affords an example of how quite different genes may produce very similar and even unique characters.

Compressed

Compressed has several features characterizing it. Dr. METZ furnishes me the information that an autosomal character, telescoped, in *virilis*, has the same peculiarities. The number of features in common is almost as large as is the case between the various notch characters. The position of compressed in the chromosome map makes it improbable that the gene for compressed can be considered as identical with the gene for telescoped. This case shows that two similar characters, which may have as many as four features in common, are not necessarily to be regarded as identical, unless confirmatory evidence is obtained.

It is not now evident what part of the *obscura* X chromosome corresponds to that of either *melanogaster* or *virilis*. The different sequence for yellow, white, and notch, together with the occurrence of several characters which are similar to, but not identical or parallel with, ones in other species, has complicated the problem exceedingly. Sufficient data to solve it are not yet available.

I wish to thank Prof. T. H. MORGAN for valuable suggestions and assistance. Dr. A. H. STURTEVANT has helped me greatly with his advice and interest and I am further indebted to him for caring for my experiments at a time when I was unable to do so. Dr. C. W. METZ very kindly turned over to me his stocks of *Drosophila obscura* including several mutant races.

SUMMARY

1. A study of *Drosophila obscura* was undertaken in order to determine how the chromosome group of this species was related to that of *D. melanogaster* and of *D. virilis*.

2. In pedigreed cultures, 28 mutations have occurred in 23 loci in the X chromosome.

3. A consideration of the linkage relations of the various mutants indicates that the total amount of crossing over in the X chromosome of

D. obscura is greater than in any other linkage group heretofore studied. A map of the X chromosome based on these linkage relations shows a total distance of about 170 units, which exceeds the length of the maps of the second or third chromosomes in *D. melanogaster* by about 60 units.

4. There is evidence that certain of the mutants obtained are identical with sex-linked mutants in other species. The strongest cases are those of notch and of white-eosin. The loci of these genes in the *obscura* X chromosome are in the central region over 60 units from either end, while the loci of the similar mutants in *D. melanogaster* are very near the end of the X chromosome.

5. It is suggested that the greater length of the X chromosome, both cytologically and genetically, as compared with the X chromosomes of *D. virilis* and *D. melanogaster*, is due to the presence of a large piece of chromosome, attached to what corresponds to the left hand end of the *melanogaster* X chromosome.

6. Any attempt to identify the sex-linked characters of *D. obscura* with those of the two other species is difficult. Several mutants in *D. obscura* which are very similar in appearance to mutants in either *D. melanogaster* or *D. virilis* do not seem to be identical. The similarly named mutants, yellow, notch, and white-eosin, are not in the same linear order in *D. obscura* and in *D. melanogaster*.

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APPENDIX

TABLE 14

Sex-linked mutations of Drosophila obscura.

NAME	CHARACTER AFFECTED	SYMBOL	LOCUS	DATE	STOCK
Pointed	Wings	P	62	January, 1920	Lethal$_1$ test
Glazed	Eyes	g_z	58.	July 1920	s_c-y cross
Beaded	Wings	b	42.	August, 1919	s- (non-disjunction)
Scutellar	Bristles	s_c	0 3	April, 1920	s_l stock
Yellow	Body color	y	0 0	March, 1920	s_l stock
Notch	Wings, life bristles, etc.	N	5.	December, 1919	Lethal$_1$
White	Eye color	w	6.	February, 1920	Test of long
Eosin	Eye color	w^e	6.	August, 1920	s_c N linkage
Vermilion	Eye color	v	14.5	January, 1920	Test of s_b
Polished	Body color	p_o		July, 1920	y s_l linkage
Singed	Bristles	s_g		October, 1920	y w linkage
Miniature	Wings	m_n		July, 1920	y L_a linkage
Reduced	Bristles	r	24.	January, 1920	Lethal$_1$
Broken	Venation, life,	B_r		December, 1919	s- cross
Rough	Eyes	r_o	36.	January, 1920	r s linkage
Compressed	Thorax, eyes, etc.	c_o		March, 1920	b s linkage
Fused	Venation	f_u	42.	January, 1920	Inheritance B_r
Ascute	Thorax	a_s	86 5	August, 1920	s_c y v linkage
Slender	Bristles	s_l	99.5	February, 1920	Test sex ratio
Short	Venation	s	107.5	1916 (METZ)	Wild
Shorter	Venation	s^r	107.5	October, 1919	Test gap
Shortest	Venation	s^s	107.5	November, 1919	b s- (non-disjunction)
Dwarf	Size	d_w		December, 1919	s- cross
Extended	Wings	e_d		April, 1920	N r_o linkage
Deformed	Thorax, eyes, wings, etc.	d_f		November, 1919	Inheritance gap
Stubby	Bristles	s_b		December, 1919	No. 553 (METZ)
Lethals	Life		Various		Any stock

TABLE 15

Two-point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		CROSSOVERS		TOTAL
$b\ N_2$	$b \times N$	0	201	118	0	319
$b\ s_l$	$b \times s_l$	28	20	26	18	92
$y\ N_2$	$y \times N$	0	129	10	0	139
$y\ w$	$y \times w$	217	305	17	14	553
	$y\ w \times +$	184	100	10	14	308
$N_2\ w$	$N \times w$	700	0	9	0	907
	$N \times w$	240	246	4	1	491
$N_2\ w^e$	$N \times w^e$	340	374	4	0	718
$N_2\ s^r$	$N \times s^r$	109	0	98	0	207
$b\ s$	$b \times s$	140	142	134	115	531
$s_c\ y$	$s_c \times y$	142	109	0	1	252
$s_c\ v$	$s_c \times v$	120	91	15	13	239
$y\ v$	$y \times v$	59	50	9	3	121
$p_o\ r_o$	$p_o \times r_o$	58	62	1	1	116
$v\ p_o$	$v \times p_o$	70	44	10	10	140
$v\ f_u$	$v\ f_u \times +$	46	32	11	20	109
$v\ B_r$	$v \times B_r$	0	380	47	0	427
$c_o\ f_u$	$c_o \times f_u$	208	140	23	2	373
$N\ v$	$N \times v$	131	0	0	8	139
$B_r\ f_u$	$B_r \times f_u$	0	106	22	0	128
$B_r\ s$	$B_r \times s$	0	28	29	0	57
$s\ d_w$	$s \times d_w$	42	61	7	4	114
$s\ s_b$	$s\ s_b \times +$	77	71	2	0	150
$s\ s_l$	$s \times s_l$	130	147	13	12	302

NOTE. In these tables the same arrangement has been followed as that used by STURTEVANT (1921). "The column headed 'Loci' shows not only the loci concerned but also their sequence. In the third and following columns classes are entered under their headings indicating the type of crossing over they represent. In every case that class which includes the individuals bearing the wild-type allelomorphs in the left-hand locus concerned is placed first, and is followed by the contrary class."

TABLE 16

Three-point linkage experiments.

LOCI	TYPE OF CROSS	NON-CROSSOVERS		SINGLE CROSSOVERS				DOUBLE CROSSOVERS		TOTAL
				Region 1		Region 2				
$P\ g_s\ v$	$P \times g_s\ v$	40	41	2	1	40	41	0	4	176
$P\ b\ v$	$P \times b\ v$	40	52	21	0	32	52	0	7	204
$P\ y\ a_s$	$P \times y\ a_s$	18	68	42	5	23	38	37	14	245
$g_s\ b\ y$	$g_s\ y \times b$	18	10	2	2	9	11	1	1	54
$g_s\ b\ v$	$g_s \times b\ v$	25	18	10	11	5	9	0	0	78
$b\ w\ s$	$b\ s \times w$	121	157	95	77	91	143	103	50	837
$b\ v\ s$	$b\ s \times v$	21	18	18	16	26	34	21	16	170
$b\ N\ s$	$b\ s \times N$	0	46	0	23	0	44	34	0	147
$b\ r\ s$	$b\ s \times r$	32	48	33	18	30	21	34	25	241
$b\ f_u\ s$	$b\ f_u\ s \times +$	62	45	32	47	49	31	38	47	351
$s_c\ y\ w^e$	$s_c\ y \times w^e$	141	166	12	3	0	2	0	0	324
$s_c\ y\ v$	$s_c \times y\ v$	109	92	0	0	27	28	0	0	256
	$s_c\ v \times y$	74	69	0	0	10	11	0	0	164
$s_c\ y\ r_o$	$s_c\ y \times r_o$	41	44	0	1	10	5	0	0	101
$s_c\ r_o\ s_l$	$s_c\ s_l \times r_o$	33	27	9	9	20	27	14	8	147
$y\ N_2\ w^e$	$y\ N \times w^e$	167	201	11	7	4	1	0	0	391
	$y\ N \times w^e$	0	569	0	22	8	0	0	0	599
	$y\ w^e \times N$	214	175	9	10	1	5	0	0	424
	$y\ w^e \times N$	0	152	14	0	0	1	0	0	167
$y\ N\ v$	$y\ v \times N$	0	162	15	0	0	11	0	0	188
$y\ v\ s_g$	$y\ v \times s_g$	133	147	22	15	3	4	0	0	324
$N_2\ r\ r_o$	$N \times r\ r_o$	0	71	29	0	11	0	0	2	113
$w\ p_o\ r$	$w\ r \times p_o$	87	49	2	1	4	4	0	0	147
$w\ m_n\ r$	$w\ r \times m_n$	216	62	16	4	23	2	2	1	326
$v\ B_r\ r_o$	$v\ r_o \times B_r$	0	77	0	7	5	0	0	0	89
$v\ r\ r_o$	$v \times r\ r_o$	91	224	16	7	10	20	3	5	376
$c_o\ a_s\ s_l$	$c_o\ a_s \times s_l$	80	49	77	47	11	13	6	8	291
$c_o\ a_s\ s$	$c_o \times a_s\ s$	91	95	98	50	27	26	28	13	428

TABLE 17

Four-point linkage experiments.

LOCI	NATURE OF CROSS	NON-CROSSOVERS		SINGLE CROSSOVERS						DOUBLE CROSSOVERS						TRIPLE		TOTALS
				1		2		3		1.2		1:3		2.3		1:2:3		
$g_s\ y\ v\ s$	$g_s\ y\ v\ s \times +$	38	33	21	21	11	3	29	22	2	7	26	26	4	5	4	5	257
$g_s\ y\ f_u\ s$	$g_s\ y\ s \times f_u$	6	11	8	3	4	2	10	6	0	2	9	2	5	2	1	3	74
$g_s\ b\ y\ v$	$g_s\ y \times b\ v$	33	16	9	6	16	16	2	1	0	1	0	3	4	3	0	0	110
$b\ v\ m_n\ f_u$	$b\ v\ f_u \times m_n$	65	32	22	38	2	1	8	34	0	0	16	5	0	0	0	1	224
$b\ v\ f_u\ s$	$b\ f_u\ s \times v$	40	33	20	22	9	29	40	33	15	8	26	24	15	17	9	1	341
$b\ v\ B_r\ f_u$	$b\ v\ f_u \times B_r$	0	29	15	0	1	0	0	12	0	3	0	17	3	0	0	1	81
$b\ c_o\ f_u\ s$	$b\ c_o \times f_u\ s$	46	37	39	47	3	0	56	40	1	3	24	31	2	0	0	1	330
$v\ r\ r_o\ f_u$	$v\ f_u \times r\ r_o$	88	120	18	2	6	13	0	2	2	1	0	0	0	1	0	0	253
$w\ s_g\ f_u\ s$	$w\ s_g \times f_u\ s$	101	65	8	0	55	11	96	53	2	3	10	4	38	7	1	2	456
$c_o\ a_s\ s_l\ s$	$c_o\ a_s \times s_l\ s$	87	29	71	41	12	0	7	1	4	7	0	2	0	0	0	0	261

TABLE 18

Summarized data for each pair of loci in the X chromosome.*

REGION	TOTAL	CROSSOVERS	PERCENTAGE OF CROSSING OVER
$y\ N_2$	1712	88	5.2
$y\ w$	1185	70	5.9
$y\ v$	1231	179	14.5
$y\ r_o$	212	39	18.4
$y\ s_c$	1268	4	0.3
$y\ b$	275	115	41.8
$N_2\ w$ or w^e	3419	37	1.1
$N_2\ v$	327	19	5.8
$N_2\ r$	113	31	27.4
$N\ b$	466	175	37.5
$w\ s_g$	456	30	6.8
$w\ s$	837	387	46.2
$w\ b$	1112	432	38.8
$v\ m_n$	224	4	1.8
$v\ p_o$	116	2	1.7
$v\ s_g$	324	7	2.2
$v\ r$	668	56	8.4
$v\ B_r$	508	55	10.8
$v\ f_u$	341	103	30.2
$m_n\ r$	326	28	8.6
$p_o\ r$	147	8	5.4
$p_o\ r_o$	140	20	14.3
$s_g\ f_u$	456	119	26.0
$r\ r_o$	742	74	10.0
$r\ f_u$	275	40	14.5
$B_r\ r_o$	89	7	7.8
$r_o\ f_u$	253	3	1.2
$c_o\ f_u$	703	35	5.0
$c_o\ a_s$	428	189	44.1
$a_s\ s_l$	552	63	11.4
$f_u\ s$	1753	826	47.2
$a_s\ s$	428	94	21.9
$s\ s_l$	302	25	8.2
$s\ s_b$	150	2	1.3
$s\ b$	531	249	46.9
$s_c\ v$	239	28	11.7
$b\ g_s$	242	39	16.1
$b\ P$	204	28	27.4
$g_s\ p$	176	7	4.0

*NOTE. The data used in the above table are comprised entirely of data in which no intermediate locus was present between the loci concerned. For instance, in a three-point cross, the direct crossover values can be calculated for the first and second, and for the second and third loci; then by ignoring the middle locus, the direct crossover can be calculated for the first and third loci from the same experiment. This third value is not included in the above table.

THE INHERITANCE OF SKI WINGS IN *DROSOPHILA MELANOGASTER*

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INTRODUCTION

While a great number of the mutant characters of *Drosophila melanogaster* have been found to follow a simple mode of Mendelian inheritance, not a few involve more or less complex factor relations. These latter have been of great value in demonstrating the generality of the phenomena involved in Drosophila studies. To those who have worked much with Drosophila the simplicity and definiteness of the results is regarded as a natural consequence of the material which has been used in the studies. The original wild type of the species occurs in nature over a very wide area, yet it is apparently monotypic in form and possesses a germinal constitution essentially identical throughout its extensive distribution.

From this relatively invariable wild type, mutations have been discovered from time to time which have evidently involved changes in specific elements in the germinal material. Since these changes have occurred either directly from the wild type or in other mutant stocks not far removed in derivation from it, they have evidently been of a kind eminently suited for genetic investigation. Of all plant and animal material, that obtained from Drosophila has most nearly approached the ideal for genetic experimentation, for it has furnished a wide array of forms which differ from a single wild type only in the simple characters which they exhibit. One need only compare this condition with that which obtains in most plants and animals, where investigations have been conducted with varieties which have long been established, and which almost invariably differ from one another in a large number of characters, and even more complexly in their germinal make-up, to realize, aside from its ease of culture and short life cycle, the unique superiority of this material for genetic research. Even in some of the more involved cases, Drosophila investigations have been relatively simple as compared with the complexity which is often exhibited in other forms. On the other hand, such typical investigations as those of ALTENBURG and MULLER (1920) on truncate wings, BRIDGES (1919) on modifiers of eosin eye color, MULLER (1918) on balanced lethals, BRIDGES and MOHR (1919) on vortex, STURTEVANT (1918) on number of spines in dichaete strains, MORGAN (1919) on notch wings, and the epoch-making investigations of BRIDGES (1916, 1917, 1919) on non-disjunction, deficiency, duplication and the like, reveal how far our conceptions of germinal structure and behavior have been determined by the results of the more complex Drosophila investigations.

Now although several investigations have been made with characters which are the result of complex factor interactions, the results of such studies have not often been as clean-cut as those from other characters, because of the variability of the forms in question. Thus, BRIDGES and MOHR (1919) point out in their analysis of vortex that while the character depends essentially upon the interaction of two genes, v_{oII} and V_{oIII}, males usually exhibit vortex only when of the composition $\frac{v_{oII}}{v_{oII}}\ \frac{V_{oIII}}{V_{oIII}}$, but among the females approximately 20 percent of those of the constitution $\frac{v_{oII}}{v_{oII}}\ \frac{V_{oIII}}{\ }$ also show a variable development of the vortex character. In addition to these complications a special relation of the character to streak was discovered; and two additional modifying genes were demonstrated which had specific effects upon its development. In most plants and animals such a complex series of interactions would be extremely difficult of precise analysis, but in Drosophila the development of a special technique makes it possible to eliminate guesswork, and to follow genes from generation to generation with perfect assurance, even though their effects are for the time being completely suppressed. Nevertheless it would seem probable that the precise analysis of other more definitely stable characters involving complex factor relations would add to the general acceptability of the results.

In the present paper the results of the investigation of a bigenic character which does not exhibit phenotypic difficulties are reported. The analysis has been carried out more completely than was necessary for a determination of the actual factor relations at work in the production of the character, because the absence of phenotypic variability in it made it possible to demonstrate the adequacy of the bigenic hypothesis in a variety of ways not possible had such variability occurred. Moreover, it is recognized that there are those who would wish to see not only the consequences of the analysis pointed out; but would also desire a full demonstration of actual experimental results in confirmation of the analysis. We have attempted, therefore, to devise critical experiments under control which would satisfy this very natural demand.

ORIGIN AND HISTORY OF SKI

The history of the ski stock runs back to October 10, 1915. On that date a quart fruit-jar containing a liberal supply of fermenting banana was exposed in the Botanic Garden of the UNIVERSITY OF CALIFORNIA in order to secure wild strains and species of Drosophila. One week later the culture was brought into the laboratory. On October 20th the banana in this jar, together with the contained larvae, was transferred to a pint milk bottle. On October 28th the culture was examined and found to contain 28 *melanogaster* and 6 *obscura* individuals. Of the 28 *melanogaster*, 4 were white-eyed males. At the time of examination it was noted that these white-eyed flies were probably the result of contamination from laboratory sources after the fruit jar had been brought into the laboratory.

Nevertheless these four white-eyed males were saved and mated in mass culture to wild-type females from a laboratory stock. Culture Cr10, thus established, produced 45 wild-type flies. From these wild-type flies two further mass cultures were raised, Cr49 and Cr50, each from five pairs of flies (table 1). The large number of ski flies secured from these cultures indicates that ski appeared originally as a segregation product. Inasmuch as the white-eyed males which were mated to females from a laboratory stock of wild-type flies were doubtless escapes from our laboratory stock of white, there is every reason to believe that ski did not derive its origin from the fresh wild stock secured in the exposed fruit jar.

A stock culture of ski was eventually established from culture Cr49, and this stock was maintained in the ordinary routine fashion. It was observed, however, that matings of ski flies often produced some wild-type individuals, but this fact was ascribed to phenotypic variation in the character. It was also observed that in stock cultures of ski it was necessary to select for ski in order to prevent "reversion" to wild-type.

TABLE 1

Original cultures from which ski flies were secured.

	WILD-TYPE		WHITE		SKI		WHITE SKI	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
Cr49..........	316	164	..	142	18	4	..	5
Cr50*..........	146	76	6	7	1	1	..	..

* The 6 white females were produced after the culture had been yielding flies for over a month, consequently they clearly belonged to a second generation produced in the same bottle.

Since ski closely resembled descriptions of the jaunty and curled mutants, which had been discovered at COLUMBIA UNIVERSITY, we obtained stocks of these flies from Professor MORGAN. Reciprocal matings of curled × ski and jaunty × ski made in June, 1916, in all cases gave wild-type F_1, thus demonstrating that ski was a distinct new character.

Up to 1919 a number of attempts were made to determine the mode of inheritance of ski, but these came to nothing on account of the failure to recognize its bigenic nature. The experiments were all carried out under the assumption that ski, like jaunty and curled, which it resembles phenotypically, was a monogenic character. The appearance of wild-type flies in cultures which should have been all ski was ascribed to phenotypic variation analogous to that which had already been reported for certain characters such as club wings. A rather extensive series of investigations designed to determine the locus of ski also gave discordant results, due to the fact that ski flies always segregated out in a much lower ratio than that expected for a simple recessive character. During this period proper stocks were not on hand in the Laboratory of Genetics of the UNIVERSITY

OF CALIFORNIA, to carry out expeditiously a precise analysis of a complex character. In 1919, however, the authors secured additional stocks of flies from COLUMBIA UNIVERSITY, and embarked upon an extensive series of experiments designed to demonstrate its mode of inheritance.

DESCRIPTION OF SKI

As stated before, ski bears a close resemblance to the jaunty and curled mutants discovered at COLUMBIA UNIVERSITY. It is in fact the form which BRIDGES and MORGAN (1919) call "California curled" in their discussion of jaunty. There is also a sex-linked jaunty, now extinct, which belongs to the same "genus of mutants." Ski is variable in expression, but if the wings are fully developed, there is never the slightest difficulty in separating it quickly and accurately from normal-winged flies. In its less extreme expression, simplex ski, it is almost identical with jaunty, and cannot be separated from it in mixed cultures. In this expression the wings are slightly, but distinctly, bent upward at the tips, the curvature usually involving only the terminal half of the wing, although some individuals do show a rather uniform upward sweep of the wings from the basal portion. The more extreme expression, duplex ski, is typically characterized by a marked curvature of the wings closely approximating curled in appearance. In this expression the wings are often so strongly recurved that the tips point directly forward. Although these two types of ski are fairly distinct, the variation in expression among them is such as to render accurate and rapid classification of mixed populations into the two ski classes out of the question. The genetic significance of the two classes will be pointed out below.

Although it would be impossible to separate ski from jaunty and curled in mixed populations with the unaided vision, there are certain associated characteristics which are revealed by magnification with a binocular which distinguish it from other, like forms. Thus in jaunty the wings are slightly thicker in texture than those of normal flies, and they are frequently corrugated transversely. In ski the texture does not seem to differ appreciably from normal, and transverse corrugations seldom occur. In curled the wings are slightly thinner than normal and have a waxy or silvery texture. Often the upper surface of the wing has a crumpled appearance very much like that of a thin piece of paper unevenly bent. Sometimes this crumpling results in a blistered appearance of the tissue of the wing. The wings of jaunty are often more strongly curved on the inner side, and they may have a slight outward slant. The outward slant is even more noticeable in ski, and it is still more striking in curled.

In jaunty and curled the wings are almost flat transversely and evenly upturned, but in curled the upper surface is somewhat convex, which may account for the crumpling of the wing tissue in this form. In curled the wings are noticeably indented on the inner margin at the end of the fifth longitudinal vein, whilst in jaunty and ski, the margin is evenly curved at this point.

The body color of jaunty is said to be slightly darker than that of wild-type flies. In our experience with jaunty strains isolated from a multiple stock, this difference, if present at all, is extremely slight. Ski flies do not show any detectable difference in body color from wild-type. The stocks of curled which we have had, however, have all exhibited a very marked dark body color, almost as dark as that of black flies. Whether this character is associated with curled has not been determined; but if it is, it will provide a ready means for identifying curled.[1] In jaunty, the posterior scutellars point straight backward as they do in wild-type, but in ski and curled these bristles point inward and laterally, crossing near the middle.

The most noticeable positive associated characteristic of ski is exhibited by the costa or marginal vein of the wing. In wild-type this vein has an even, outward curve in prolongation of the first longitudinal vein. In typical ski, however, the costa exhibits a sharp infolding, making a deep "crimp" in the proximal portion of the marginal cell. This "crimp" is merely a partial persistence of the deep fold which occurs in this region of the wing pad of newly-emerged flies. It is an upward and inward fold in the wing, and apparently is more pronounced the more strongly recurved the wing. In simplex ski the "crimp" is often very slight, but still detectable. It is not present in jaunty or curled flies, consequently it is possible to make a rough separation of a mixed population on the basis of the presence or absence of this "crimp" in the wings. Along with this "crimp" there is more or less "buckling" all along the length of the costa, and this is also more pronounced in the more strongly recurved wings. The tissue of the marginal cell is crumpled somewhat as a consequence of the irregularity of the costa.

Although there is a certain amount of variation in the expression of ski, it is never of such a nature as to confuse separation of ski from wild-type. The least-marked expression of ski is perfectly definite and readily separable from normal without recourse to magnification. Its ready and unmistakable separation from wild-type makes it a very favorable character for investigation.

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[1] BRIDGES reports that this dark color is due to the same gene that produces curled, or, less probably, to a very closely linked modifier.

BIGENIC NATURE OF SKI

During 1918 at a time when it seemed possible that we would be unable to perpetuate ski ourselves or to do further work with it, a culture of it was sent to the Zoölogical Laboratory of COLUMBIA UNIVERSITY. In 1919 when we resumed work with it it soon became evident that we would need appropriate stocks of dominant characters in order to analyze it expeditiously. Accordingly we wrote to Professor MORGAN for cultures of the dominant mutants, bar, star, and dichaete. In our letter we called attention to our work to date, which showed that with ski and wild-type segregants from ski the following series of results had been secured:

Wild-type × wild-type gave uniformly wild-type flies only.
Ski × ski gave either all ski or 3 ski : 1 wild-type.
Ski × wild-type gave either all ski or 1 ski : 1 wild-type.

We also pointed out that by continued sister × brother matings we had been able to establish a stock breeding true for ski. This statement, however, later proved to be incorrect, for the stock subsequently produced wild-type flies. In reply Doctor STURTEVANT, to whom Professor MORGAN referred this letter, stated that he and Doctor BRIDGES had also performed some preliminary experiments with ski. They were able to confirm our results and in addition to point out that F_1 star dichaete males from matings of star dichaete × ski back-crossed to wild-type segregants from ski gave two types of results, namely,

(a) 1 star dichaete : 1 star : 1 dichaete : 1 ski
(b) 1 star dichaete : 1 star : 1 dichaete : 1 wild-type

On the basis of these results they outlined a hypothesis, which we shall refer to as the bigenic hypothesis, to the effect that ski depends upon a difference from wild-type in two genes, one a dominant gene located in the second group, and the other a recessive located in the third. To the second-chromosome gene we assign the symbol, $S_{i_{II}}$, and to the third, $s_{i_{III}}$, and throughout this paper we shall use the abbreviations, S_i and s_i, respectively, for these symbols. Neither of these genes alone produces any difference from wild-type. For the production of ski it is necessary that s_i be homozygous, and that S_i be either in the heterozygous or homozygous condition. STURTEVANT and BRIDGES recognized the occurrence of $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$ flies, for in this case S_i, although dominant, exhibits no recessive lethal effect such as is shown by most of the dominant mutants in Drosophila.

Since we have found the bigenic hypothesis fulfilled at all points, it seems well in the interest of clarity of presentation to reverse the customary procedure, and to present the general results of the analysis before giving the experimental data. We have accordingly constructed table 2, which gives a convenient summary of all the ratios of wild-type : ski which may be secured from different matings involving the genes, S_i and s_i and their normal allelomorphs. We have summarized the results of these forty-five different types of reciprocal matings below by grouping those which give the same ratio of wild-type : ski together and stating the ratios in terms of 16 for the sake of more ready comparison:

27 give 16 wild-type : 0 ski
1 gives 14 wild-type : 2 ski
1 gives 13 wild-type : 3 ski
5 give 12 wild-type : 4 ski
1 gives 10 wild-type : 6 ski
6 give 8 wild-type : 8 ski
1 gives 4 wild-type : 12 ski
3 give 0 wild-type : 16 ski

Such a variety of ratios would be very puzzling in any bisexual form in which the methods of analysis had not been developed to the perfection found in Drosophila. In Drosophila, however, it is possible to test this entire array of matings under perfect control.

TABLE 2

The theoretical results of the 45 possible types of reciprocal matings involving the genes S_i and s_i and their normal allelomorphs. The genotypes of the parents are given along the top and the phenotypes in the left-hand column. The genotype of each phenotype may be obtained by reference to the genotype of the same number. The ratios are stated throughout in terms of wild-type : ski.

PHENOTYPES	TYPE NUMBERS AND GENOTYPES								
	1	2	3	4	5	6	7	8	9
	— —	$\frac{S_i}{\;}$ —	$\frac{S_i}{S_i}$ —	— $\frac{s_i}{\;}$	— $\frac{s_i}{s_i}$	$\frac{S_i}{\;}\ \frac{s_i}{\;}$	$\frac{S_i}{\;}\ \frac{s_i}{s_i}$	$\frac{S_i}{S_i}\ \frac{s_i}{\;}$	$\frac{S_i}{S_i}\ \frac{s_i}{s_i}$
9 duplex ski	1 : 0	1 : 0	1 : 0	1 : 1	0 : 1	1 : 1	0 : 1	1 : 1	0 : 1
8 wild-type	1 : 0	1 : 0	1 : 0	3 : 1	1 : 1	3 : 1	1 : 1	3 : 1	
7 simplex ski	1 : 0	1 : 0	1 : 0	3 : 1	1 : 1	5 : 3	1 : 3		
6 wild-type	1 : 0	1 : 0	1 : 0	7 : 1	3 : 1	13 : 3			
5 wild-type	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0				
4 wild-type	1 : 0	1 : 0	1 : 0	1 : 0					
3 wild-type	1 : 0	1 : 0	1 : 0						
2 wild-type	1 : 0	1 : 0							
1 wild-type	1 : 0								

Some of the consequences of this hypothesis are very curious. Thus, duplex ski, $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$, mated to wild-type, $\frac{\ }{\ }\ \frac{s_i}{s_i}$, gives F_1 ski, $\frac{S_i}{\ }\ \frac{s_i}{s_i}$, and F_2, 3 ski : 1 wild-type. Here ski behaves as a simple dominant to wild-type, the wild-type segregants obtained in F_2 breed true when mated *inter se*, and of the ski individuals one-third are homozygous for ski when tested against this wild-type race and the remaining two-thirds are heterozygous. However, when individuals of this same duplex ski race are mated to members of a different wild-type race, $\frac{S_i}{S_i}\ \frac{\ }{\ }$, F_1 is wild-type, $\frac{S_i}{S_i}\ \frac{s_i}{\ }$, and F_2 consists of 3 wild-type : 1 ski. In this latter case the F_2 ski segregants breed true when mated *inter se*, but of the wild-type segregants, one-third are homozygous for wild-type and the other two-thirds are heterozygous for ski. In this case wild-type behaves as a simple dominant to ski, the exact reverse of the behavior described in the first cross.

There are curious linkage relations which also depend upon the bigenic nature of ski. Thus sepia, $\frac{\ }{\ }\ \frac{s_e\quad\ }{s_e\quad\ }$, mated with duplex ski, $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$, gives wild type F_1, $\frac{S_i}{\ }\ \frac{\quad\ s_i}{s_e\quad\ }$, and in F_2 9 wild-type : 3 ski : 4 sepia : 0 sepia ski. This ratio does not differ strikingly enough from the 2 : 1 : 1 : 0 ratio normally expected from a mating between two simple recessive mutants whose genes lie in the same group, to be detectable with certainty under ordinary conditions. It would, therefore, unquestionably be interpreted as a ratio indicating that ski is a simple recessive character dependent upon a gene located in the third group. But if we should take a sepia race of the constitution, $\frac{\ }{\ }\ \frac{s_e\ s_i}{s_e\ s_i}$, which would not differ in appearance from the one used in the first cross, our F_1 would be ski, $\frac{S_i}{\ }\ \frac{s_e\ s_i}{\quad\ s_i}$, and F_2 would consist of 9 ski : 3 wild-type : 3 sepia ski : 1 sepia, indicating with equal certainty that ski is not a member of the third group. It would not profit us much to follow out this analysis more completely at this point. We have accomplished our purpose, if we have laid a foundation upon which the results of the experiments may be discussed expeditiously.

The duplex ski flies referred to in the description of ski are those of the genetic constitution, $\frac{S_i}{S_i}\ \frac{s_i}{s_i}$, while simplex ski flies are those of the heterozygous genotype, $\overset{S_i}{-}\ \frac{s_i}{s_i}$. The term ski is used when the ski class consists of both duplex and simplex flies, in other cases the appropriate term, duplex or simplex, is used. A race of the constitution, $-\ \frac{s_i}{s_i}$, indistinguishable in appearance from ordinary wild-type, we call an s_i-wild-type race; one of the constitution, $\frac{S_i}{S_i}\ -$, also indistinguishable from ordinary wild-type, we call an S_i-wild-type race.

EXPERIMENTAL METHODS

The experimental methods employed in Drosophila studies are doubtless familiar enough to most investigators to make a detailed account of them unnecessary in this paper. Since some deviations from the practice employed at COLUMBIA UNIVERSITY were, however, necessary on account of the equipment on hand, we have thought it desirable to describe briefly the particular methods which we have used. It has been found necessary to give careful attention to the conditions of experimentation in order to secure satisfactory results with ski. It should be distinctly understood that these methods are not recommended in preference to those which have been outlined in detail by BRIDGES (1921), for a small amount of comparative work which we have done indicates that somewhat better results may be secured by those methods and that less time is necessary for the care and examination of cultures.

General culture methods

Standard wide-mouthed homeopathic vials of 8-dram capacity (equivalent to 29.6 cc) were used for culture bottles. The vials were prepared for use by inserting a strip of towel paper and a cotton plug in them, and they were then thoroughly sterilized in a hot-air sterilizer. Fermenting banana was used for food. Ripe bananas were cut into sections 0.5 to 1.0 cm thick, covered with water in a fruit jar, and steam-sterilized for about fifteen minutes. After cooling, a small quantity of Fleischmann's yeast was added to the jar, and then fermentation was allowed to go on at room temperature for about twenty-four hours before using the material. As cultures were needed they were prepared by simply inserting about 5 cc of fermenting banana into each vial. This amount of banana was found sufficient under proper conditions to supply food for as many as 200 flies from the time of hatching through their entire life-cycle. Cultures were kept in a small incubator room maintained at a constant temperature of 25°C by means of a thermostatically-controlled electric heating unit.

The use of small vials instead of the larger half-pint milk bottles has been found to give fairly satisfactory results. Naturally fewer flies can be grown in such vials, so that in order to raise large progenies from a single mating, it is necessary to transfer the parents to successive vials at short intervals. When this procedure is followed, the full progeny of a pair of flies may be secured. In one case a pair of flies which was transferred successively at five-day intervals yielded 919 flies in eight sub-cultures. The method of frequent sub-culturing increases the amount of time and attention necessary for the work, but it has some compensations in the greater ease of examination of cultures.

Maintaining stocks

In Drosophila investigation, it is usually necessary to synthesize and maintain a certain number of stock strains. In our work these stocks were studied and examined until they were known to be established for their characters, after which they were merely examined occasionally in order to determine whether they were continuing true to type. Stocks were continued merely by separating out two pairs of flies from an old culture for the establishment of a new one. New cultures thus established were ordinarily sub-cultured at four or five day intervals. By this method an adequate supply of any stock was always on hand, and danger of losing weak stocks or those possessing low viability was reduced to a minimum.

Procedure with experimental cultures

Cultures upon which counts were made were always established with a single pair of flies. Virgin females were isolated from vials which had previously been emptied of all flies. The individual pedigree of every culture was known absolutely. The parents of experimental cultures were transferred five successive times at two-day intervals to new sub-cultures. Two-day sub-cultures rarely produce more than 100 flies each, so that the amount of food present is adequate for the development of all the larvae. Larval competition seemed to be of no serious consequence, as the results will show, when sufficient food for all was present in the vial; but when the food supply was insufficient, or the food became dry, serious distortion of ratios would often occur through partial elimination of weak classes or those having a slightly longer period of larval development. That the food supply actually was sometimes inadequate, particularly in cultures which had given an unusually high yield, was shown by the emergence of pale, under-sized flies towards the end of the period of examination.

Examination of flies

Cultures were examined every second day during the period of emergence. Flies were etherized in the usual manner for examination. After the flies had been separated into the appropriate classes counts were recorded on form blanks conveniently devised for the purpose, one blank being used for each sub-culture. In case of classification for microscopic characters, such as star and spineless, a binocular was used. It was necessary to exercise care throughout to retain cultures until a full count had been secured, otherwise the proportion of ski flies would be low.

CHARACTERS EMPLOYED IN THE ANALYSIS

To avoid complication the number of stocks employed in the analysis was kept at a minimum. For the sake of completeness and ready reference they are enumerated here with brief descriptions of them. They were all obtained from the Zoölogical Laboratory of COLUMBIA UNIVERSITY, so that identity with their stocks is assured.

Star, gene *S*, locus II–0.0, is a dominant mutant characterized by an irregular jumbling of the facets of the eye. It is lethal when homozygous. It requires proper illumination and magnification for separation from wild-type, and care must be exercised to avoid confusing it with other like malformations of the eyes. A full account of this form, together with an illustration, is given by BRIDGES and MORGAN (1919).

Black, gene *b*, locus II–46.5, is an incompletely recessive mutant having a greenish-black coloration of the body and wings. Wild-type and heterozygous flies are treated as one class, readily and accurately separable from black without magnification. Its locus is the base of reference for the entire second chromosome. It is illustrated in color and fully described by BRIDGES and MORGAN (1919).

Sepia, gene s_e, locus III–25.8, is a recessive form characterized by a distinct dark-brownish coloration of the eyes. It is also readily separable from wild-type flies without magnification. Although it has long been known, no complete account of it has yet been published.[2]

Dichaete, gene *D*, locus III–38.5, is a dominant mutant with outwardly spread wings and a reduced number of dorso-central and scutellar bristles. Like star, it is lethal when homozygous. It is readily separable without magnification by means of the wing character. For examination of reduction in number of bristles magnification is, of course, necessary. Dichaete has been described and illustrated by STURTEVANT (1918).

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[2] The locations of the third-chromosome mutants are taken from a map published by BRIDGES (1921).

Spineless, gene s_s, locus III–54.2, is a recessive mutant which exhibits extreme reduction in the size of all body spines. The authors have always used magnification in separating it, although it is possible for a skilful investigator to classify it with the unaided vision. Spineless has been used in a large number of investigations, but no thoroughgoing account of it has yet been published.

None of these characters interferes with any other one or with ski to such an extent as to preclude the use of any combinations involving it. Thus, dichaete and ski produce a fly, dichaete ski, which exhibits a combination of the two characters. The wings are spread out from the body as in dichaete, but instead of being straight or slightly curved downward, they are strongly recurved at the tips. Similarly dichaete and spineless do not conflict, for if all the dorso-central and scutellar spines should be absent on account of the action of dichaete, it would still be possible to recognize dichaete spineless flies by the effect of spineless on the other spines of the integument. There are, therefore, no special difficulties involved in the handling of these characters in the same experimental cultures aside from those which have been mentioned for each character.

EXPERIMENTAL PROOF OF THE BIGENIC HYPOTHESIS

The most convenient method of determining the group to which a new gene belongs is that developed by BRIDGES and MORGAN (1919) in which the dominant characters star and dichaete are used as markers of the second and third groups, respectively. For example, if sepia be crossed with star dichaete, F_1 consists of four classes, namely,

1 star dichaete : 1 star : 1 dichaete : 1 wild-type..................(1)

If we now back-cross F_1 star dichaete males, there being no crossing over in them, to sepia females, we obtain the following segregation ratio:

1 star dichaete : 1 star sepia : 1 dichaete : 1 sepia..................(2)

Since sepia does not assort independently of dichaete, it must belong to the third group. If we perform the same series of experiments with black, the back-cross gives the following distribution:

1 star dichaete : 1 star : 1 black dichaete : 1 black..................(3)

Here black does not assort independently of star, and it must, therefore, belong to the second group. In case the character belongs to neither the second nor the third group, it would assort independently both of star and of dichaete, and it would then be a question of the first or fourth group. The sex distribution would indicate whether or not it belonged to the first group. If the character showed no sex linkage, it would be assigned by exclusion to the fourth group. Should the character in question prove dominant, appropriate modifications in the matings may be made for the determination of the group.

When duplex ski is crossed with star dichaete, F_1 conforms to ratio (1) above. When F_1 star dichaete males are mated to duplex ski females, the following distribution is obtained:

1 star dichaete : 1 star ski : 1 dichaete : 1 ski....................(4)

This ratio conforms to ratio (2) above, and might be taken to indicate that ski is a recessive character determined by a gene located in the third group.

Section 1 of table 3 contains the results of five cultures which conform to this distribution. The star dichaete males of the four D cultures were secured from a mating of duplex ski ♀ × star black sepia dichaete ♂, while that of culture 92 was from star dichaete ♀ × duplex ski ♂. The duplex ski individuals were taken from a proven stock established in the manner outlined below.

We have, however, pointed out above that it is possible to isolate a race of wild-type flies recessive to ski. The fact that this race is recessive to ski would naturally be interpreted as due to the fact that it bears the recessive third-group gene upon which the distribution obtained in ratio (4) depends. The conclusive evidence that a second-chromosome dominant is necessary for the production of ski is afforded by back-crosses of F_1 star dichaete males of the same constitution as those used in the above experiment to females of the wild-type recessive to ski. The results secured conform to the following distribution:

1 star dichaete : 1 star : 1 dichaete : 1 ski........................(5)

It will be observed, therefore, that a single second chromosome derived from the duplex ski race is sufficient to produce the ski character when the third-chromosome gene is homozygous. When it is heterozygous, however, as is shown by the dichaete classes of both ratios (4) and (5), the character is not produced whether the individuals be heterozygous or homozygous for the second-chromosome gene.

TABLE 3

Results of matings devised to prove the bigenic nature of ski.

SECTION NUMBER AND DESCRIPTION		*SD*	*SSi**	*S*	*D*	*Si*	+	TOTAL
1. P_1 $SD\times$ duplex S_i Duplex S_i♀♀ × F_1 SD♂♂ (Back-cross)	D2	59	48	......	34	36	......	177
	D3	63	41	......	52	51	......	207
	D4	64	41	......	53	43	......	201
	D5	54	38	......	27	36	......	155
	92	45	43	......	26	45	......	159
	Total	285	211	......	192	211	......	899
2. P_1 $SD\times$ duplex S_i s_i-+♀♀ × F_1 SD♂♂ (Back-cross)	93	84	......	99	88	67	......	338
	94	59	......	48	79	55	......	241
	95	112	.....	100	75	84	......	371
	97	90	.....	70	101	84	.....	345
	Total	345	......	317	343	290	......	1295
3. P_1 $SD\times$ duplex S_i Simplex S_i♀♀ × F_1 SD♂♂ (Back-cross)	124	48	25	28	50	51	.....	202
	125	57	39	33	84	69	......	282
	Total	105	64	61	134	120	......	484
4. P_1 $SD\times s_i$-+ Duplex S_i♀ × F_1 SD♂ (Back-cross)	121	37	56	......	44	35	......	172
5. P_1 $SD\times s_i$-+ Simplex S_i♀♀ × F_1 SD♂♂ (Back-cross)	122	102	36	30	87	42	46	343
	123	95	60	43	94	27	54	373
	132	113	46	54	132	43	58	446
	Total	310	142	127	313	112	158	1162
6. P_1 $SD\times s_i$-+ s_i-+♀♀ × F_1 SD♂♂ (Back-cross)	113	77	......	96	89	......	87	349
	131	87	......	77	114	......	96	374
	Total	164	.....	173	203	......	183	723

* The symbol S_i is also used to denote the *character* ski as it occurs in tables and matings, and the symbol s_i-+ is used to denote s_i-wild-type in matings.

Section 2 of table 3 contains results of back-crosses of F_1 star dichaete males of the same constitution as those used in section 1 to s_i-wild-type females. The star dichaete males used in these cultures were derived from the same source as the one used in culture 92, and the s_i-wild-type females were taken from a stock culture.

The fact that ski is not a monogenic character dependent upon a single recessive third-chromosome gene, as might be concluded from ratio (4), may be demonstrated directly by mating *inter se* the star ski segregants from appropriate cultures. The results from three pairs of such matings of star ski segregants from culture 92 are recorded in table 4. If the character were simply determined by a third-chromosome gene, the progeny should all be ski in the ratio of 2 star ski : 1 ski. The actual results, however, demonstrate the presence of a class of star flies. The interpretation of the proportion of star flies actually obtained must, of course, take into account absence of crossing over in the males, the lethal effect of star when homozygous, and the percentage of recombinations of S and S_i in the females. Assuming for this last item a value of 26.6 percent, which is that obtained in experiments for the determination of the locus of S_i, expectation calls for the following ratio:

TABLE 4

Star ski segregants from culture 92 mated inter se.

	S	SS_i	S_i	TOTAL
128	9	132	66	207
144	13	111	69	193
156	15	183	90	288
Observed	37	426	225	688
Expected	*61*	*398*	*229*	*688*

86.7 star ski : 50.0 ski : 13.3 star. .(6)

This ratio is in fair agreement with the actual results secured, although there is a deficit in the star class.

From these results we conclude that ski differs from wild-type in two pairs of genes. The third-chromosome gene is recessive to its normal allelomorph and the one in the second-chromosome is dominant. There is no recessive lethal effect associated with the dominant gene, as is true of most of the dominant mutant genes in Drosophila. The consistent results secured further demonstrate that the ski genotypes are invariably ski in appearance, consequently we have no difficulties of somatic variation to deal with in working with it.

ESTABLISHMENT OF DUPLEX SKI STOCKS UNDER CONTROL

The accurate analysis of ski and the location of its genes depends upon the establishment of ski stocks known absolutely to be duplex. In our original material simplex and duplex ski flies were usually mixed together, so that a great variety of results was secured from experiments with them.

The constitution of a ski fly may be determined by crossing it to members of a wild-type stock which has descended from ski parents. If the ski fly is simplex the progeny of such a mating will consist of equal numbers of ski and wild-type flies, whilst if it is duplex, the population will consist entirely of ski flies. It is not usually feasible, however, to test females before making the desired matings with them, consequently matings have been devised in which the progeny fall into diagnostic classes; i.e., classes the individuals of each of which belong to a known genotype. By double-mating males which prove to be duplex ski with star dichaete females it is possible to be assured of the production of F_1 star dichaete males of the constitution, $\frac{S}{S_i}\ \frac{D}{s_i}$. Such star dichaete males mated to duplex ski females give ratio (4) above, a practical demonstration of which is afforded by the results contained in section 1 of table 3. If, however, a selected female should happen to be simplex ski, the distribution among the progeny would conform to a different ratio, namely,

2 star dichaete : 1 star : 1 star ski : 2 dichaete : 2 ski . . . . (7)

The splitting of the star class into two sub-classes, star and star ski, definitely sets this ratio apart from the previous one.

An actual demonstration of conformance to ratio (7) is given in section 3 of table 3. The star dichaete males in this particular instance were not secured in the direct manner employed in the previous cases, but were taken from cultures in which the star dichaete males were known to be of the desired constitution. In culture 125 the star dichaete male was taken from culture 92, and the one used in culture 124 was derived from an uncounted culture with a history identical with that of culture 92. The simplex ski female of culture 124 was taken from culture 95, that of culture 125 from culture 93, in both of which cultures, as may readily be determined, all the ski segregants must have been simplex in constitution.

At the risk of being tedious the analysis must be carried further in order to point out some relations not shown in the above illustrations. For example, the necessity for determining the constitution of the original ski males and for selecting only those which prove to be duplex, may be demonstrated by considering the consequence of employing simplex ski males in the above manner. When a star dichaete female is mated to a simplex ski male the F_1 star dichaete segregants are of two genotypes, namely, $\frac{S}{S_i}\ \frac{D}{s_i}$ and $\frac{S}{}\ \frac{D}{s_i}$. The results of mating males of the former genotype to duplex ski females have been discussed and illustrated above, but males of the latter mated to duplex ski females will give progenies conforming to precisely the same distribution, except that the ski segregants are all simplex! Ratio (4), therefore, is not assurance of the production of a duplex ski class unless the star dichaete parent is known to have been $\frac{S}{S_i}$ $\frac{D}{s_i}$. As an experimental demonstration of this case, we have only the single culture recorded in section 4 of table 3. The star dichaete male was secured from s_i-wild-type ♀ × star dichaete ♂, both parents from stocks, and the duplex ski female used in the back-cross was also from a stock culture.

Results from matings of this latter type of star dichaete male with simplex ski females and with s_i-wild-type females are contained in sections 5 and 6, respectively, of table 3. The star dichaete males in section 5 were obtained from the same culture which produced the one used in culture 121, and the simplex ski females were again taken from cultures 93 and 95. The results here are clearly in agreement with the expected ratio:

2 star dichaete : 1 star : 1 star ski : 2 dichaete : 1 wild-type : 1 ski. . . (8)

The star dichaete males used in section 6 were also derived from the same culture as those used in sections 4 and 5, and in both cases they were mated to s_i-wild-type females from stock. No ski flies were produced. The results of this mating have been included merely for the sake of completeness. In agreement with expectation, the results conform to the general F_1 star dichaete ratio:

1 star dichaete : 1 star : 1 dichaete : 1 wild-type. . . . . (9)

The results should, however, be compared with those contained in section 2. In the controlled production of duplex ski, it would obviously be feasible, if desired, to test a mixed lot of F_1 star dichaete males by mating them to s_i-wild-type females rather than to test the original males, but a double mating can not be avoided at some stage in the procedure.

In a few cases we have made use of the method of testing both males and females by mating them to s_i-wild-type flies. In testing a ski female in this fashion she is first mated to an s_i-wild-type male. The male is left with her until she begins to produce eggs after which he is removed, and the female sub-cultured without the male until exhausted of all fertile eggs. She may then be mated to a duplex ski male which has previously himself been tested with an s_i-wild-type female. Those cultures, the female parents of which gave only ski progeny in the test with s_i-wild-type, may then be retained for the establishment of duplex ski stocks. While this method is more direct than the one first outlined, it is rather uncertain in operation on account of the difficulty of keeping the females alive long enough to make the two matings. The work of NONIDEZ (1920) may be taken to indicate that the method is satisfactory provided that sufficient care is exercised to exhaust the tested females of all fertilized eggs before they are again mated. The objection that one can not always be sure that the female no longer contains a few spermatozoa from the previous mating may be met by reversing the procedure, i.e., mating females to known duplex ski males first and then testing them against s_i-wild-type males. In any case where this method of establishing the duplex ski stocks has been used specific mention of it is made in the text.

There are a number of other ways, some of which will be indicated in what follows, of securing duplex ski flies under control; but those described have been used in most of the work. The work, of course, was greatly facilitated after the morphological differences between simplex and duplex ski flies had been discovered. When extremely recurved ski individuals with pronounced "crimps" in the wings are used for parents throughout, it is practically certain that they will prove duplex in constitution. It is, therefore, necessary to establish fewer cultures when such selection is practiced. However, it is not safe to rely entirely upon morphological expression as an index of germinal constitution, consequently all duplex ski stocks which have been used in critical experiments have been established under rigid control.

MATINGS GIVING VARIOUS RATIOS OF WILD-TYPE TO SKI

In view of the adequacy of the experiments designed to determine the genetic basis of ski, and the location of its genes, it is not necessary to demonstrate separately all the relations which might be shown by the various types of matings involving the genes S_i and s_i and their normal allelomorphs. We have been content to perform only a few such experiments as opportunity arose in the prosecution of what we considered the more important experiments.

Simplex ski flies mated *inter se* should produce 3 ski : 1 wild-type. This is the only mating of ski × ski which gives any wild-type segregants. The ideal method of demonstrating this ratio is to mate duplex ski with s_i-wild-type flies to produce simplex ski, and then to mate these F_1 simplex ski flies *inter se*. We have been content, however, in table 5 to present results from ski × ski matings of parents selected from our original stock while it was still throwing wild-type segregants. Several of these matings gave nothing but ski offspring, hence they must have been either of the type simplex ski × duplex ski or duplex ski × duplex ski. All cultures which gave any wild-type segregants are included in table 5. Wild-type flies are, as usual, in excess of the expected proportion of 25 percent. In these particular cultures the condition may have been somewhat aggravated by the fact that only two five-day sub-cultures were established under each culture number, but later results secured by the two-day sub-culture method show no particular increase in the proportion of ski flies realized, to that expected. If we set the survival of wild-type at 100 percent, then the ski class in these cultures is, on the average, 80.8 percent of expectation.

TABLE 5

Results from simplex ski mated inter se.

	S_i	+	TOTAL
Sk3	95	49	144
Sk5	34	13	47
Sk10	122	45	167
Sk11	76	14	90
Sk29	136	70	206
Total	463	191	654

An exact reversal of the foregoing ratio, namely, 3 wild-type : 1 ski, is accomplished by mating wild-type individuals of the constitution, $\frac{S_i}{S_i}\ \frac{s_i}{\quad}$, *inter se*. The *modus operandi* in this case was as follows. From duplex ski ♀ × star black sepia dichaete ♂ an F_1 star male was taken and back-crossed to a duplex ski female. The progeny of this mating consisted of 55 star, 52 star ski, 32 ski, and 49 wild-type flies. Here the wild-type flies must necessarily be $\frac{S_i}{S_i}\ \frac{s_e\qquad}{\qquad s_i}$. The results of matings of four pairs of wild-type flies are given in table 6. The influence of better conditions of experimentation is strikingly reflected in the total yields and ratios obtained from these four cultures. In this series each culture number was represented by five two-day sub-cultures. Although the ski segregants were all duplex in this case the attained values reach a percentage of 86.6 when compared with non-ski as 100 percent. In fact the number of ski individuals is slightly in excess of that of sepia, although these two classes should, theoretically, be present in equal numbers.

From the above cultures four test matings were made of ski × ski. All produced only ski descendants. This set of experiments was also partly instituted in order to secure a race of flies homozygous for S_i, but with the normal allelomorph of s_i. Since there is no crossing over in the male and not in excess of 15 percent of recombination of s_e and s_i in the female, it is evident that at least 85 percent of the sepia segregants in the above cultures are of the desired constitution. We started the desired stock, therefore, by mating sepia segregants *inter se.* This stock, which we call the S_i-sepia stock, has been carried on for over a year in mass cultures without producing a single ski segregant. Consequently its constitution may safely be considered as that desired.

TABLE 6

Wild-type $\left(\frac{S_i}{S_i}\ \frac{s_e\qquad}{\qquad s_i}\right)$ *mated inter se.*

	+	s_e	S_i	TOTAL
C7	260	113	111	484
C8	282	126	111	519
C9	241	76	119	436
C10	269	92	80	441
Total	1052	407	421	1880

The possession of S_i-sepia and s_i-wild-type stocks placed us in a position to reconstruct ski from two stocks breeding true for the normal wing type. Accordingly we made up a culture of S_i-sepia ♀ × s_i-wild type ♂, and one of the reciprocal. The two cultures yielded 535 wild-type flies. Two F_2 cultures were started with F_1 flies from the former of these two cultures. The results are recorded in table 7. It is clear from these results that ski flies may be obtained by crossing two stocks indistinguishable in appearance from wild-type. The expected ratio, 9 wild-type : 4 sepia : 3 ski, is not, however, well realized. As a matter of fact, ski flies occur in a proportion of only 35.8 percent of expectation, assuming as before 100 percent as the value of the non-ski classes. It is probable that this result is due to the unusually high production of these cultures, which favored elimination of the later-emerging ski flies by over-crowding in the culture bottles. The discrepancy in the ratio is practically confined to the ski class, for sepia flies occur in 97.1 percent of expectation, setting the wild-type class at 100.

We have also had occasion to make crosses of F_1 wild-type flies from duplex ski × wild-type, therefore, $\frac{S_i}{-}\ \frac{s_i}{-}$, in constitution, back to duplex ski flies. In this case the back-cross should give a ratio of 1 wild-type : 1 ski. The results from three cultures are given in table 8. They show a survival value of ski of 84.7 percent compared with the wild-type class.

TABLE 7

P_1, S_i-sepia ♀ × s_i-wild-type ♂. F_1 wild-type mated inter se.

	+	*Se*	*Si*	TOTAL
153	385	157	55	597
154	333	153	30	516
Total	718	310	85	1113

The cultures are also interesting because they show such a marked decrease in total production as compared with the other series of cultures reported in this section. A lowered productivity appears to be associated constantly with our duplex ski races, and to a somewhat lesser extent with simplex ski, so that some extra attention must often be given the stocks in order to keep them in a thriving condition.

TABLE 8

P_1, duplex ski ♀ × wild-type ♂. F_1 duplex ski ♀ × wild-type ♂.

	+	*Si*	TOTAL
B1	76	72	148
B3	92	86	178
B4	101	70	171
Total	269	228	497

A considerable number of other matings could be readily devised to illustrate interesting relations of ski contrasted with various wild-type genotypes. It is not, however, necessary to go through the complete set of them in order to demonstrate adequately the mode of inheritance of ski. What is important, however, in this connection, is a realization of the fact that every mating may be made under perfect control, so refined are the modes of analysis in Drosophila. It is not necessary in Drosophila, as is often the case in other forms, to conjecture the genotypes involved in a particular mating from the results of that mating. It is perfectly possible and feasible to know before the matings are made what the genotypes of the parents are, and what results should be secured from them.

DETERMINATION OF THE LOCUS OF SKI-II

The determination of the loci involved in a bigenic character presents no particular difficulty, save that the establishment of appropriate stocks is a tedious and troublesome task. At the outset we decided to employ the three-point method of locating each gene and to carry out the four necessary complementary back-crosses in order to balance the inviability of ski, which we at first thought was rather marked.

For the determination of the locus of S_i, star and black were used as reference points. They were the only suitable second-chromosome genes which we had represented in our stocks; and, fortunately for the accuracy of the determinations, S_i was found to lie between them. As far as we know, no two better reference points could have been chosen, because there is absolutely no interference in the expression of the three characters in any combination. In experiments for the location of S_i it is necessary to have all stocks homozygous for s_i so that ski will appear whenever S_i is present. Since S and S_i are both dominant genes, the back-cross takes in each case the form of F_1 star simplex ski ♀ × black-s_i ♂. In order to perform the four complementary back-crosses, the eight types of flies listed below were necessary:

Star-s_i $= \frac{S \qquad\qquad}{\qquad\qquad} \; \frac{s_i}{s_i}$

Star ski $= \frac{S \; S_i \qquad}{S_i \qquad} \; \frac{s_i}{s_i}$

Star black-s_i $= \frac{S \qquad\qquad b}{b} \; \frac{s_i}{s_i}$

Star ski black $= \frac{S \; S_i \quad b}{S_i \quad b} \; \frac{s_i}{s_i}$

Ski $= \frac{S_i \qquad}{S_i \qquad} \; \frac{s_i}{s_i}$

Ski black $= \frac{S_i \quad b}{S_i \quad b} \; \frac{s_i}{s_i}$

Black-s_i $= \genfrac{}{}{0pt}{}{b}{b} \; \genfrac{}{}{0pt}{}{s_i}{s_i}$

s_i-wild-type $- \frac{s_i}{s_i}$

It is not necessary to establish stocks of all these different types, for obviously each of the four different star stocks will give the corresponding one of the non-star types by segregation. It was found more satisfactory, however, in some cases to have actual stocks of the non-star types on hand rather than to depend upon segregation in star stocks for them. Pertinent details in the establishment of the stocks will be included in the discussion of the individual back-cross experiments.

Results from the $\frac{S}{S_i\, b}$ back-cross are recorded in table 9. The F_1 star ski flies were produced from the mating, star-s_i ♀ × ski black ♂. The star-s_i stock had been secured from an original mating of star dichaete ♀ × duplex ski ♂. From such a mating the F_1 star dichaete flies, mated *inter se*, produce, among other classes, a star ski class, the members of which are mostly simplex for ski. Such F_2 star ski flies, mated *inter se*, produced the desired star-s_i flies as an F_3 segregation product. The ski black stock was established by mating together the F_2 ski black individuals from an original cross of duplex ski ♀ × black ♂. In this case the F_2 ski black segregants are all simplex and the F_3 progenies conform to the ratio, 1 duplex ski black : 2 simplex ski black : 1 black-s_i. From among the ski black segregants individuals of both sexes were selected which had the most strongly recurved and conspicuously "crimped" wings, and tested by mating them to black-s_i flies. In each case the progenies consisted entirely of ski black flies. The tested ski black females were then exhausted of all fertile eggs and subsequently mated to tested ski black males for the establishment of the duplex ski black stock. While there is an element of uncertainty, as we have stated before, in the testing of females, no difficulty can arise from it here, because the production of F_1 (star and non-star) ski progeny from star-s_i ♀ × ski black ♂ is absolute assurance that the F_1 star ski females are of the proper constitution for use in the

TABLE 9

P_1, star-s_i ♀ × ski black ♂. Back-cross, F_1 star ski ♀ × black-s_i ♂.

	S	$S_i b$	$SS_i b$	+	Sb	S_i	SS_i	b
189A	122	94	41	53	14	31	18	6
189B	107	82	27	36	21	20	5	2
189C	99	60	39	41	15	26	16	1
189D	56	55	12	28	3	14	5	1
Total*	384	291	119	158	53	91	44	10
	107	82	27	36	21	20	5	2

* The totals carried forward to table 13 are stated on the second line. They represent the results of culture 189B only.

back-cross test. A black-s_i stock was established at the same time from the F_3 black-s_i flies.

An examination of the data from these four cultures shows that three of them suffer from marked disproportions especially in the star black and ski and in the star ski and black classes. Culture 189B is free from these discrepancies. Since like irregularities do not appear in subsequent data, except in one culture, it would seem that these three cultures have not yielded normal results for the back-cross, but indicate the existence of some undetermined disturbing agent. They are, therefore, not included in the total carried forward to table 13, even though most of the results of their irregularity would be swamped in the data of that table.

The results of the $\frac{S\ \ S_i}{b}$ back-cross are recorded in table 10. The F_1 star ski flies were produced from the mating, star ski ♀ × black-s_i ♂. The star ski stock was established from the same line of cultures which was the source of the star-s_i stock. It was not established as a duplex ski stock, but was merely maintained by selecting star ski flies for the production of each successive generation. In order to produce the F_1 females for the series of cultures included in table 10, selected star ski males were mated to black-s_i females. F_1 star ski females were then taken from a culture which produced nothing but star ski and ski flies.

TABLE 10

P_1, star ski ♀ × black-s_i ♂. Back-cross, F_1 star ski ♀ × black-s_i ♂.

	SS_i	b	Sb	S_i	SS_ib	+	S	S_ib
190A	141	100	50	55	25	23	3	3
190B	161	166	63	71	30	34	8	6
Total	302	266	113	126	55	57	11	9

The results of the $\frac{S\ \ S_i\ \ b}{\ }$ back-cross are contained in table 11. The F_1 star ski females were produced from the mating, star ski black ♀ × s_i-wild-type ♂. For the establishment of star ski black stock, star ski black males were taken from culture 190A. They must obviously have been of the constitution $\frac{S\ \ S_i\ \ b}{b}\ \frac{s_i}{s_i}$. They were mated to stock ski black females, whereby the progeny was divided equally between two classes, star duplex ski black and simplex ski black. The former, mated *inter se*, were the foundation for the star ski black stock. The s_i-wild-type stock had been established in the usual manner by taking the wild-type segregants from simplex ski flies mated *inter se*.

The results of the $\frac{S \quad b}{S_i}$ back-cross are recorded in table 12. The F_1 star ski females were produced from the mating, star black-s_i ♀ × duplex ski ♂. The establishment of the star black-s_i stock was effected by simply mating star black segregants from culture 190A *inter se*. The duplex ski stock was the original stock from which all the stocks mentioned in this account were derived.

TABLE 11

P_1, star ski black ♀ × s_i-wild-type ♂. Back-cross, F_1 star ski ♀ × black-s_i ♂.

	SS_ib	+	S	S_ib	SS_i	b	Sb	S_i
195A	145	176	60	64	35	32	2	3
195B	97	112	37	31	12	12	1	3
195C	71	69	37	30	15	18	..	3
195D	73	79	30	32	12	7	..	2
195E	99	104	30	39	18	22	2	..
195H	48	76	34	29	14	6	..	1
195K	71	73	32	26	13	16	2	..
195L	97	108	51	38	25	15	2	3
Total	701	797	311	289	144	128	9	15

TABLE 12

P_1, star black-s_i ♀ × ski ♂. Back-cross, star ski ♀ × black-s_i ♂.

	Sb	S_i	SS_i	b	S	S_ib	SS_ib	+
196A	71	83	28	36	12	14	2	1
196B	72	57	35	26	21	14	11	4
196C	90	76	38	41	17	27	1	1
196D	13	61	21	61	64	4	1	31
196G	50	34	16	18	2	5	1	..
196H	89	95	43	30	23	23	1	4
Total*	385 372	406 345	181 160	212 151	139 75	87 83	17 16	41 10

* The totals carried forward to table 13 are stated on the second line. They do not include the results of culture 196D.

In culture 196D there are marked disproportions in all the four pairs of classes, which indicates that the results belong to a different class of data from those given by the other five cultures of this series. Here again the nature of the disturbance was not determined, but the results from this culture are not included in the totals carried forward to table 13.

We have been content to outline the mode of procedure which was actually employed in the experiments, leaving to the reader's ingenuity the problem of determining for himself how it might have been modified to avoid establishment of so many complex stocks. Such modification is perfectly feasible, if advantage be taken of the exact knowledge of the genotype of every individual in a given back-cross population.

A summary of the results secured from the four complementary back-crosses, excluding the aberrant cultures which have been noted in the discussions of the corresponding tables, is contained in table 13. Similar tables containing the percentages of recombinations for each of the several cultures were prepared but they are not included in this account. Aside from the aberrant cultures, the amount of variation shown in the values calculated from the separate cultures is no greater than that met with normally. For the 4845 flies included in table 13 direct computation gives recombination values of 26.6 percent for S and S_i, 13.6 for S_i and b, and 37.1 for S and b. The direct value given by BRIDGES and MORGAN (1919) for S and b is 37.9, based on 16,507 flies. The agreement, therefore, is satisfactory. The coincidence of crossing over, based upon the totals of table 13, is 43.8 percent, calculated as follows:

TABLE 13

Summary of the results of the four complementary back-cross experiments giving data on the relations of S, Si and b.

COMBINATIONS	——	—|——	——|—	—|—|—	TOTAL	RECOMBINATION PERCENTS		
						S, S_i	S_i, b	S, b
$\dfrac{S}{S_i \quad b}$	189	63	41	7	300	23.3	16.0	34.7
$\dfrac{S \quad S_i}{b}$	568	239	112	20	939	27.6	14.1	37.4
$\dfrac{S \quad S_i \quad b}{}$	1498	600	272	24	2394	26.1	12.4	36.4
$\dfrac{S \quad b}{S_i}$	717	311	158	26	1212	27.8	15.2	38.7
Total	2972	1213	583	77	4845	26.6	13.6	37.1

$$\frac{77\times4845\times100}{1290\times660}=43.8 \text{ percent}$$

In determining the probable locus of S_i from these data it is still necessary to allow for a certain amount of undetected double crossing over in the two sections of the chromosome under consideration. Approximate corrections can be made by proper substitution in the formula proposed by BRIDGES and MORGAN (1919), namely,

$$x^2-\frac{x}{c}=-\frac{o}{2c}$$

In this equation, x is one-half the corrected distance, c is the coincidence, and o is the observed recombination value, all stated as decimal fractions. For the probable value of the coincidences in these two sections we have taken values assumed by BRIDGES and MORGAN for similar sections of the chromosome. Thus they correct their dachs-black data from a direct value of 17.8 percent to 18.5 on the basis of an assumed value of 50 percent for coincidence. This region is comparable to our S_i-b region, the direct value of which, 13.6 percent, must then be corrected to 14.1, assuming the same value for coincidence. Similarly they assign a value of 30 percent for the coincidence in the star-dachs region, the direct value of which, 27.3 percent, is comparable to our S-S_i value. S-S_i, then, with a direct value of 26.6 percent, is corrected to 27.8 on the basis of an assumed coincidence of 30 percent. In view of the low coincidence value actually obtained from our data, objections may be raised to the high values which have been used in correcting them; but, since the values are proportional, this fact probably does not result in any appreciable error in the calculations. We then have $27.8+14.1=41.9$ as the value for the star-black distance. The mean value which BRIDGES and MORGAN employ for this distance, based on all pertinent data for the second-chromosome genes, is 46.5, a value which exceeds our determination by 4.6 percent. This discrepancy must be taken into account in the final assignment of a proper locus for S_i in terms of the map which they have constructed for the second-chromosome genes. If the difference is divided in proportion to the lengths of the two sections involved, the final value for S_i becomes 30.8, a position slightly to the right of dachs. S_i does not, therefore, occupy any hitherto unrepresented region of the second chromosome. The ease of classification of the character and its dominance make the mutant one to be considered in genetical studies in which this region is followed. The fact that all the flies used in the final matings of such experiments must be homozygous for s_i adds a few generations or a few cultures to the preparatory work, which is not a serious handicap in Drosophila.

DETERMINATION OF THE LOCUS OF SKI-III

Preliminary investigations indicated that the loci for dichaete and spineless would provide suitable reference points for the determination of that of s_i. Accordingly stock cultures of duplex ski spineless and s_i-spineless were made up in the customary manner. The establishment of an s_i-spineless race presents no difficulties, because any spineless segregants from ski parents will necessarily be of this type. In the establishment of duplex ski spineless, however, it is necessary, after ski spineless flies are obtained, to follow the method previously outlined in this paper in order to assure ourselves of the duplex character of the stock. The duplex ski spineless stock has been maintained for nearly two years in mass cultures without producing a single wild-type fly.

The details pertinent to the performance of each of the four types of mating are discussed below. It seems desirable, however, to describe at this point the general method which was used in the prosecution of the experiments. In the preceding set of experiments for the determination of the locus of S_i, ten cultures were started for each type of mating, a total of forty cultures for the entire series of experiments, but only twenty of them were successful. The failures resulted apparently mostly from unfavorable bacterial growths, which are often transferred from bottle to bottle with the parents, if they once become established, and to the necessity for using ski females in the back-crosses. In the S_i series of cultures no attempt was made to replace unsuccessful cultures. This accounts for the discrepancy in the numbers of cultures secured in the different back-crosses. In the s_i series of cultures, it was decided at the outset to replace all unsuccessful cultures until results from ten cultures were secured from each back-cross. In order to do this with the minimum amount of labor, stock cultures were established from which virgin females of proper constitution for use in back-crosses could be isolated at any time. Since in every case the back-cross takes the form, dichaete ♀ × duplex ski spineless ♂, these stocks were maintained by successive matings of dichaete males with appropriate females. The following list enumerates the various matings which were necessary for maintaining these stocks:

(1) $\dfrac{\qquad\qquad}{}$ ♀ × $\dfrac{D \quad s_i \quad s_s}{}$ ♂

(2) $\dfrac{\qquad\qquad s_s}{}$ ♀ × $\dfrac{D \quad s_i}{\qquad s_s}$ ♂

(3) $\dfrac{s_i \qquad\qquad}{s_i \qquad\qquad}$ ♀ × $\dfrac{D \qquad\qquad s_s}{s_i}$ ♂

(4) $\dfrac{s_i \qquad s_s}{s_i \qquad s_s}$ ♀ × $\dfrac{D \qquad\qquad}{\qquad s_i \quad s_s}$ ♂

The last stock in this list was never established in this manner, but it is included for the sake of completeness. The actual experiments with this combination were carried out in a different manner, which is fully described below. It is understood that S_i is present in none of these stocks. Each of the stocks obviously segregated in each generation for the two classes of flies represented by the parents, and once established they could easily be maintained by mating virgin non-dichaete females to dichaete males. When it was desired to make a back-cross test, it was merely necessary to take out virgin dichaete females from the appropriate stock and mate them to duplex ski spineless males. The manner in which the original stocks were established is described in connection with the account of the experiments in which they were used.

The series of cultures for the $\frac{D}{s_i \quad s_s}$ combination was carried out first. It differs in method from the other series of cultures in that the gene, S_i, was here introduced from the female side, so that the actual matings for the linkage determinations were of the type, $\frac{S_i}{S_i}\ \frac{D}{s_i \quad s_s}$ ♀ × —— $\frac{s_i \quad s_s}{s_i \quad s_s}$ ♂. This type of mating is of some additional interest because both parents are non-ski in appearance, yet on crossing them the progeny is distributed in a ratio of 1 non-ski : 1 ski.

In order to secure dichaete females of the above type, a duplex ski spineless female was mated to a star dichaete male. An F_1 star dichaete male from this mating was then back-crossed to a duplex ski spineless female, whereby a progeny conforming to the following ratio was obtained:

1 star dichaete : 1 star ski spineless : 1 dichaete : 1 duplex ski spineless.

Here all the non-star flies must be homozygous for S_i. Dichaete females were, therefore, isolated and mated to s_i-spineless males with the results contained in table 14. This method is needlessly complex, for, given an s_i-spineless stock, the obvious mode of operation would be to mate individuals of it to dichaete and then to back-cross the F_1 dichaete females to duplex ski spineless males. The method adopted was followed merely in order to show that the determinations could be made satisfactorily in this manner.

The series of cultures for the $\frac{D \quad s_i \quad s_s}{}$ combination, included in table 15, has the following history. A dichaete ski spineless male from culture

H11 (cf. table 14) was taken as the starting point. He must obviously have been of the constitution, $\frac{S_i}{}\ \frac{D\ s_i\ \ s_s}{s_i\ \ s_s}$. It was necessary for the establishment of the proper stock, (1) in the list given above, to get rid of the second chromosome bearing S_i, and the third containing s_i and s_s. In order to do this the selected dichaete ski spineless male was out-crossed to a star female. The progeny secured from this mating consisted of the usual four classes (ratio (1)), but the star dichaete flies might be either $\frac{S}{S_i}\ \frac{D\ \ s_i\ \ s_s}{}$ or $\frac{S}{}\ \frac{D\ \ s_i\ \ s_s}{}$. A star dichaete male from this culture was then mated to a black sepia female (the black and sepia characters have no significance here) and following this mating star dichaete males were out-crossed for four consecutive generations to stock wild-type females until it was desired to use the stock thus established for linkage determinations. Star was finally eliminated by simply selecting dichaete males for the continuation of the stock. The dichaete flies of these latter generations could have been only $\frac{D\ \ s_i\ \ s_s}{}$ in composition. The cultures of table 15 were established from such dichaete females mated to duplex ski spineless males.

TABLE 14

Results of back-crossing dichaete $\left(\frac{S_i}{S_i}\ \frac{D\qquad\quad}{s_i\ \ s_s}\right)$ *females to s_i-spineless males.*

	D	S_is_s	DS_is_s	+	Ds_s	S_i	DS_i	s_s
H11	193	199	9	12	22	21	3	2
H12	159	197	12	4	31	23	1	2
H13	144	128	3	9	22	11	..	3
H15	63	62	6	2	11	15	..	1
H16	51	36	2	7	10	5	1	..
H17	152	137	5	10	17	13	..	..
H18	138	161	10	4	19	14	2	2
H19	176	175	8	14	28	23	2	3
H20	50	55	3	5	11	9	..	4
H21	112	113	5	7	16	18	1	4
Total	1238	1263	63	74	187	152	10	21

While it is not absolutely necessary to have females free from S_i in these experiments, it is nevertheless desirable not to introduce this gene from the female side in order to avoid the production of duplex ski segregants, which are probably of lower viability than simplex ski flies. In matings carried out in the manner described, all segregants will obviously be heterozygous for S_i, thus avoiding any difficulties which might arise, if there were differences in this respect.

The series of cultures for the $\frac{D\quad s_i}{s_s}$ combination was followed out in the same general manner as that for the one just described. The starting point was a dichaete ski male, obviously $\frac{S_i}{\ }\ \frac{D\quad s_i}{s_s}$, from culture H21 (cf. table 14). He was out-crossed to a stock star female, and then star dichaete males were out-crossed in consecutive generations to wild-type females until the original second and third (non-dichaete) chromosomes were replaced. As a matter of fact these matings were carried on for seven generations, after which the stock was maintained ready for use at any time by mating dichaete males in successive generations to spineless females. Dichaete females were isolated from this stock from time to time and mated to duplex ski spineless males until the desired number of successful matings had been secured. The results are recorded in table 16.

TABLE 15

Results of back-crossing dichaete $\left(\frac{D\quad s_i\quad s_s}{\ }\right)$ *females to duplex ski spineless males.*

	DS_iS_s	+	D	S_iS_s	DS_i	S_s	DS_s	S_i
N13	97	107	3	1	14	5	..	..
N14	140	173	4	4	14	17	1	..
N15	140	250	10	11	20	20	2	1
N16	57	128	3	1	1	12	1	1
N17	70	114	4	4	8	4	..	1
N18	161	202	6	5	20	16	3	..
N19	136	199	4	6	18	17	3	1
N20	135	275	11	8	14	25	1	..
N21	112	134	6	9	5	17	..	1
N22	133	173	6	3	21	12	1	..
Total	1181	1755	57	52	135	145	12	5

The starting point of the $\frac{D\quad s_s}{s_i}$ combination was a dichaete spineless male, obviously $\frac{S_i}{\ }\ \frac{D\quad s_s}{s_i\quad s_s}$, from culture H12 (cf. table 14). He

TABLE 16

Results of back-crossing dichaete $\left(\frac{D\ \ s_i}{s_s}\right)$ *females to duplex ski spineless males.*

	DS_i	s_s	Ds_s	S_i	DS_is_s	+	D	S_is_s
6	220	260	5	12	17	24	1	1
7	242	323	12	12	18	31	..	2
8	246	356	9	9	25	32	..	1
9	143	165	13	10	14	30	2	3
10	150	180	6	3	11	22	1	..
13	261	289	12	7	28	26	1	3
42	153	157	4	7	17	21	1	1
50	267	330	17	8	38	26	4	1
51	118	124	3	3	16	10	..	..
74	67	117	..	6	7	10	2	1
Total	1867	2301	81	77	191	232	12	13

was mated to a star female from stock, and then star dichaete males were mated in successive generations to wild-type females until the stock was free from the original S_i and s_i-s_s chromosomes, after which the stock was maintained ready for use by mating dichaete males to s_i-wild-type females. Dichaete females from this stock were isolated for the establishment of the first eight cultures of table 17.

TABLE 17

Results of back-crossing dichaete $\left(\frac{D\ \ s_s}{s_i}\right)$ *females to duplex ski spineless males.*

	Ds_s	S_i	DS_i	s_s	D	S_is_s	DS_is_s	+
35	155	155	2	6	15	17	..	..
39	161	162	6	9	26	9	..	..
40	89	101	1	4	10	13	1	1
56	152	164	18	19	24	21	4	2
57	132	136	10	13	21	21	2	2
58	134	172	16	16	25	13	1	1
59	79	70	..	2	5	8	..	..
60	140	195	9	17	23	24	..	2
177	304	266	11	17	29	31	2	3
179	223	209	11	15	31	21	..	2
180	328	246	8	24	35	39	3	3
Total	1897	1876	92	142	244	217	13	16

This stock was carelessly permitted to run out before the desired number of successful cultures had been secured from it. The females of the last three cultures of table 17 were, therefore, derived from a different source. A spineless female was mated to star dichaete male, both from stock. An F_1 star dichaete female was then mated back to a spineless male, and from the progeny a dichaete spineless male was selected and mated to an s_i-wild-type female. Dichaete females produced from this mating were then mated to duplex ski spineless males for the establishment of the last three cultures.

A summary of the data obtained from these four sets of cultures is contained in table 18. In calculating the final values for the locus of s_i we have used the totals given at the foot of the table. This method is equivalent to an assignment of weights proportional to the number of individuals counted for each combination, but it appears preferable to all other possible ones because it calls for a minimum amount of manipulation of the raw data obtained from the experiments. It may be noted in passing, however, that other methods of computation do not materially alter the values recorded in table 18. Thus, if each set of cultures is given equal weight, the linkage values become 4.8, 10.4, and 13.8 instead of 4.7, 10.3, and 13.7, respectively, and if computations are based on non-ski classes only, following BRIDGES's (1921) suggestion for calculations involving a character of inferior viability, the values become 4.9, 10.4 and 13.9, which again differ only slightly from the values secured by direct computation.

TABLE 18

Summary of the results of the four complementary back-cross experiments giving data on the relations of D, s_i and s_s.

COMBINATIONS	——	—|——	——|—	—|—|—	TOTAL	RECOMBINATION PERCENTS		
						D, s_i	s_i, s_s	D, s_s
$\dfrac{D\;\;s_i\;\;s_s}{\quad}$	2936	109	280	17	3342	3.8	8.9	11.6
$\dfrac{D\qquad}{s_i\;\;s_s}$	2501	137	339	31	3008	5.6	12.3	15.8
$\dfrac{D\;\;s_i\quad}{\quad s_s}$	4168	158	423	25	4774	3.8	9.4	12.2
$\dfrac{D\quad s_s}{s_i\quad}$	3773	234	461	29	4497	5.8	10.9	15.5
Total	13378	638	1503	102	15621	4.7	10.3	13.7

In fitting these data to those which have already been presented, we have taken BRIDGES's values, dichaete = 38.5 and spineless = 54.2, as representing the most probable values for our two reference points. The D-s_i section should, therefore, be 15.7 units in length, instead of 15.0 as given by our data. It seems unnecessary to correct these short distances for coincidence, although we are working in the region of highest coincidence in the chromosome. Consequently a simple correction based on the division of the difference in proportion to the lengths of the sections involved gives a final value of 43.4 for the locus of s_i in terms of BRIDGES's scale of values.

An examination of the data for s_i on the basis of coincidence discloses the existence of very high values throughout the series. The summarized data of table 17 give a coincidence value of 134.2 percent, when theoretically a very low value should be secured for such a short section of the chromosome. If the value is computed by taking the average of the values of the separate cultures, as suggested by WEINSTEIN (1918), a coincidence of 131.5 percent is secured, a value not differing significantly from the preceding one. We thought that errors in classification might have been responsible for the high values obtained, but an examination of the data from this standpoint has not led to the detection of any. We have, therefore, no definite explanation to offer for the high coincidence values secured in these cultures, but they seem to be real values not dependent upon errors of classification.

An interesting question is here raised as to the relation between s_i and c_u, the gene for curled. GOWEN (1919) places curled 3.7 units to the right of dichaete, but his mean value for the distance between dichaete and spineless is only 10.4, whereas later determinations raise this value to 15.7. If we correct his value for dichaete to curled proportionally, it becomes 5.6, which would place the locus of curled at 44.1, as compared with 43.4 for s_i. It might be possible, therefore, as far as these data are concerned, for s_i and c_u to be allelomorphic, although the fact that a wild-type F_1 is secured from duplex ski × curled argues strongly against this interpretation. We have also found that black flies of the constitution, $\frac{S_i\quad b}{S_i\quad b}\ \frac{s_i\qquad}{\qquad c_u}$, have wild-type wings; but we have not been able to secure final proof of non-allelomorphism through the establishment of a ski-curled race of the constitution, $\frac{S_i}{S_i}\ \frac{s_i\quad c_u}{s_i\quad c_u}$. Obviously, in view of the short distance between s_i and c this would be a difficult task, hardly worthy of the effort required. Some preliminary experiments carried out with this object failed of success, doubtless because they were not extensive enough to yield the desired results.

VALUATION OF SKI

It has already been pointed out that ski flies are always readily separable from those with wild-type wings, and that flies genotypically ski are always of that phenotype. Ski is, therefore, available for use in any case where the contrast is between wild-type and ski wings, or for any mutant form involving other than wing characters. With some other wing characters such as curved and dichaete, it may also be used with a fair degree of accuracy, although an occasional misclassification is probably unavoidable. With more-marked wing characters, it probably cannot be used. For example, we have found that with miniature it produces simply a crumpling of the wing unsuitable for accurate classification.

The viability of ski depends intimately on cultural conditions. Typically members of the ski classes appear somewhat later than those of non-ski classes in the same cultures, so that care must be taken to retain cultures until all of the flies have emerged. Examination of individual sub-culture records shows that ski flies had hardly begun to emerge in many instances before the non-ski flies had practically all appeared. It would seem reasonable to suppose that in the event of unfavorable conditions in the medium, particularly such as restrict food supply, the later portions of the count would fail to appear, thus giving rise to discrepancies in the ski classes such as have been secured occasionally in the actual results. That these discrepancies are not indicative of corresponding gametic aberrancies is clearly indicated by the fact that often they did not occur, and the proper class relations have always been secured in analytical crosses.

A quantitative examination of the amount of disproportion in the ski classes shows that it is subject to wide fluctuations. Some figures have already been presented of the relation of ski observed to ski expected where the segregation was relatively simple; i.e., where there are only a few classes in the progeny. Here the percentage of survival ranges from 35.8 to 90.0 in different experiments. The data are not extensive enough or of the right kind to determine the relative survival value of simplex and duplex ski. They are, moreover, open to the objection that the wild-type flies are of different genotypes. While the evidence indicates no striking differences in viability between different wild-type genotypes; nevertheless exact relations have not been determined, and they would require a very extensive series of experiments.

A comparison of ski and non-ski frequencies in table 3, on the basis of non-ski observed = 100 percent of expectation, gives a survival value of 90.2 percent for ski, taking all the results together. A similar computation from the data of the S_i series of back-cross experiments gives a survival value of 93.6 percent, where equal numbers of non-ski and ski are expected. The s_i series of back-cross experiments gives a survival value of 85.4 percent. In this last series of cultures, a higher average yield per culture was secured. We have often observed that cultures giving a moderate number of flies conformed more closely to expectation than those in which the yield was unusually high. We may conclude that a survival value of 90 percent may be secured under reasonably favorable conditions.

As will be readily understood from the above accounts, the usefulness of ski for instructional purposes depends almost entirely upon the cultural methods adopted. If these are good, then ski furnishes unique material for demonstrations of factor relations of what might be termed the second order of difficulty, but if good cultural conditions cannot be provided, very marked discrepancies may occur in the ratios.

DISCUSSION OF RESULTS

Some questions of no little theoretical importance are raised by the origin of ski and its mode of inheritance. We have pointed out in our discussion of its origin that in all probability no wild strain entered into the cultures from which it was finally secured, but that it came from hybrid cultures derived from our laboratory wild-type and white-eyed stocks. It might, therefore, have represented merely a recombination of genes in these two stocks, assuming that one contained S_i and the other s_i, in the same manner that we have actually obtained ski from two stocks with wild-type wings. But individuals of these two stocks had been crossed frequently in order to demonstrate the mode of inheritance of the white-eye character, and no ski segregants had ever been observed. At the present time we do not have these two stocks in their original condition, for we have often reëstablished our white stock from out-crosses to other forms and we have unfortunately not been careful to retain our wild stock in an unbroken line, but have occasionally reëstablished it from wild sources. It is, therefore, impossible to determine by direct test what the original constitution of these two stocks may have been. Assuredly, however, the first appearance of ski in our cultures was due to segregation rather than to original mutation, as is shown by the number of ski individuals secured in the first cultures, by the fact that two parallel cultures produced the new form, and by the further *a priori* difficulty of assuming the simultaneous occurrence of two mutations in the same cultures with such relations to each other. It seems certain that the mutations in both of the loci had occurred long previous to the actual appearance of the character, how long we can not even conjecture from our data.

It must be admitted that the manner of origin of ski and its mode of inheritance, considered superficially, lend some support to the oft-repeated contention that new characters arise by recombination rather than by actual mutation. While there are few geneticists who uphold this hypothesis in the extreme form in which LOTSY (1916) has developed it, nevertheless there are some who contend that undue stress has been laid upon the origin of new characters by mutation, and that many so-called mutations are nothing more than complex segregation phenomena. We have been led to give this matter particular attention through numerous discussions with the HAGEDOORNS,[3] who, while granting the occurrence of some "loss mutations," are disposed to credit the majority of cases of mutation to segregation. It is perfectly feasible to build up a plausible argument around this hypothesis, and it is doubtless true that the evidence in many cases may equally well be interpreted in either way. Thus in its simplest form, we may assume that two genotypes, in general $AAbb$ and $aaBB$, exist, which are nearly, if not quite, identical in appearance. Upon hybridization an F_1 is produced which is like the parents in appearance, assuming that the AB phenotype is also identical with Ab and aB; but in the F_2, if the double recessive, $aabb$, represents a new form, one in sixteen of the individuals will be of a different type. In populations of ordinary size, the new form would appear in such small numbers that one would naturally be inclined to regard it as a mutant form, and subsequent tests with either parent form would strengthen this belief, for in both cases a simple monohybrid ratio would be secured. Should either of the parent forms have been discarded, in view of their phenotypic identity, it would be impossible to determine the true significance of the origin of such a "mutant." The criticism that often mutations have not been subjected to adequate test matings from this standpoint appears to us a valid one.

A similar line of argument is possible for ski. Assuming that we had at the beginning two strains of wild-type flies, one containing S_i and the other s_i, hybridization would result in the production of some ski individuals in F_2. They might appear in much below the normal ratio of 13 wild-type : 3 ski, as we have shown by direct tests of this mating. If, now, we had first established ski in a pure-breeding stock by continual selection, before seeking to determine its genetic significance, and in the meantime had discarded one of our original parent stocks, ski would behave as a simple recessive in case we had retained the S_i stock, or as a simple dominant in case the other stock had been retained. In either case the origin of ski might logically have been ascribed to mutation in a single locus of whichever parent stock had been retained, and the possibility of detecting the true significance of its origin would have been lost. The recombination hypothesis applies, therefore, as well and as simply to the origin of dominant characters as to that of recessive ones.

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[3] A full discussion of their position is contained in HAGEDOORN and HAGEDOORN Vorstheuvel LA BRAND (1921).

But while we have been led from our results with ski to point out these theoretical possibilities, we do not believe they constitute a valid explanation of the origin of the many mutations in Drosophila. In the first place we have direct evidence to the contrary from the study of ski itself. It is assumed in Drosophila that the wild type is not a conglomerate of different genotypes making up a single phenotype, but that its phenotypical uniformity, wherever found, is a manifest of a corresponding genotypic homogeneity. In the work with ski it has been necessary to use a large number of mutant stocks, presumably derived from a variety of sources, yet there seems to be no escape from the conclusion that all these stocks were uniform in constitution with respect to S_i and s_i, possessing neither of them but their normal allelomorphs. Had it been otherwise, it would be difficult to account for the consistent results which have been secured. It seems to us that the same line of argument applies to all the other simple mutant characters of Drosophila, whether dominant or recessive. They have been recombined in every conceivable fashion; they have been derived from a great variety of sources; and a great variety of different stocks has been kept on hand; yet they have always behaved in a manner consistent with the hypothesis that they are the results of actual point-mutation. Moreover, direct attempts to secure new forms by hybridization of wild strains derived from distinct sources have been uniformly disappointing. New forms are apparently as often produced by stocks which have been carried on for numerous generations in the laboratory without crossing as in those which are of hybrid origin. STURTEVANT (1921) has recently summarized and discussed the evidence on this point, not only for *melanogaster*, but also for several other species, consequently it is unnecessary to recite the details here. The only logical conclusion seems to be that, while the possibility of producing new forms by hybridization must be recognized, particularly in work with the polymorphic species of domesticated plants and animals, yet in Drosophila the genetic constitution of the various stocks is so well and completely known as to preclude any general application of this hypothesis to it. It does not seem to matter, as far as the validity of these arguments is concerned, whether one subscribes to the theory of presence and absence or to the alternative one of actual change in the composition of the gene itself.

The mode of inheritance of ski has many analogies in other forms, so that any arguments which may be advanced for it may be applied by extension to such cases. In general any character relation such that a given form has two identical forms opposed to it one of which is dominant and the other recessive fulfills the conditions of parallelism. Thus CLAUSEN and GOODSPEED (1921) have recently demonstrated the existence in *Nicotiana Tabacum* of two genetically distinct red-flowering forms, one of which is dominant to pink and the other recessive. The inheritance of aleurone color in maize provides other parallel cases, for there is a white form dominant to purple and at least three recessive white forms are known which differ in a single gene from purple. Similarly in fowls reports show that the white of the White Leghorn is dominant to colored plumage while that of some other breeds, such as the Silky, White Plymouth Rock and the White Wyandotte, is recessive. In cattle a dominant white has long been known, and recently DETLEFSEN (1920) has described a recessive albino condition. In this last case the two whites are distinguishable, so that the parallelism is not as close as in the other cases. Many other instances of the same kind could be cited, but it is our purpose merely to point out a few parallel cases and not to catalogue all of them.

It is not always a simple matter to determine whether or not two genetically distinct characters, which are nearly, if not quite, identical phenotypically, may not possess some distinguishing morphological features. In the case of the red-flowering tobaccos, the authors comment at some length upon this difficulty. In describing and contrasting the various members of the "jaunty genus of mutants" in Drosophila, it is possible to enter into minute details with confidence, because it is practically certain that the residual genotypes in the various forms are identical, so that any differences among them are in all probability the effects of the mutant genes themselves. It is otherwise in the tobaccos, where such a detailed description of the difference in expression of the characters would be largely meaningless on account of the impossibility of determining without comprehensive tests whether the differences are dependent upon the existence of diverse residual genotypes or upon the effects of the particular genes under consideration. In such cases it is practically necessary to synthesize lines having the same residual genotypes before accurate conclusions can be drawn regarding the significance of slight character differences existing among the genotypically distinct members of a given phenotype. Here again we cannot refrain from calling attention to the distinct superiority of Drosophila for genetic investigations.

For those of us who are working largely with long-established cultivated forms, it is interesting to speculate upon the theoretical difficulties which may beset the investigator in the analysis of genetic phenomena in a given species. Thus if we had given us an original population consisting of jaunty, curled and ski, what a tangled skein we would have had to unsnarl! And yet we have no reason to believe that such complexities do not occur in our polymorphic species, whether wild or domesticated, and that they are not the cause of the apparent discrepancies which are met with in the literature of plant and animal genetics.

SUMMARY

1. Ski, a new wing character in *Drosophila melanogaster*, is shown to be genetically distinct from jaunty-X, jaunty, and curled, previously discovered mutant characters which it resembles phenotypically.

2. Ski is a bigenic character resulting from a change in two genes of the wild-type fly; one, $S_{i\mathrm{II}}$, a second-chromosome dominant, and the other, $s_{i\mathrm{III}}$, a third-chromosome recessive.

3. Neither $S_{i\mathrm{II}}$ nor $s_{i\mathrm{III}}$ produces any somatic change except when acting with the other.

4. The locus of $S_{i\mathrm{II}}$, based on counts of 4845 flies in complementary back-crosses with S and b as reference points, is placed at II-30.8.

5. The locus of $s_{i\mathrm{III}}$, based on counts of 15,621 flies in complementary back-crosses with D and s_s as reference points, is placed at III-43.4.

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ALCOHOLISM AND THE GROWTH OF WHITE RATS

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INTRODUCTION

The data for this paper were collected in connection with experiments undertaken with the primary purpose of studying possible methods of modifying and controlling heritable characters, especially of a psychological nature. Attention is here directed to the immediate effect of alcohol fumes upon the weight of the treated animals, as well as upon the weight of their descendants. It is fully recognized that weight is an exceedingly complicated character, the result of the interaction of a great number of influences in which environmental factors and nutrition play a large part. However, under the experimental conditions of the laboratory, these last influences are largely equalized for all the animals and even if it is impossible to make definite interpretations of the channels by which the final effects upon weight are brought about, the conclusions in themselves may be of some value. PEARL (1917, p. 171) made this statement: "Clinical experience has abundantly demonstrated that body-weight changes, in spite of their rough and inexact character, furnish an index of general metabolic conditions and changes, which is by no means to be despised." The results here presented indicate that, instead of causing any direct demonstrable genetic change, the treatment has had a definite physiological effect upon the treated animals and, perhaps through this, has acted as a selective agent upon genetic differences that were already present.

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[1] Many of the data upon which this paper is based were recorded by Miss E. M. VICARI who has taken an active part in carrying out the experiments and in whose charge the whole work was left during the author's absence in 1917 and 1918. The charts were constructed by Mr. LAURENCE H. SNYDER, who has carried through a large part of the statistical work involved.

METHODS

Full details of the relationships of the rats used, the methods of breeding and the treatment, have already been published (MacDowell 1921) so it will not be necessary to repeat these details at this time. However, a full understanding of the nature of the controls employed is vital to the proper evaluation of the results here recorded. The females in a litter were divided into two groups at random: those to be treated and those to be used as controls; in like manner the males in a litter were divided into two similar groups. The matings were all between full brothers and sisters from the same single pair of grandparents as well as from the same parents; treated rats were mated in all cases with treated rats and controls with controls. In the following generations the controls were always the offspring of the controls in the preceding generations, and they were raised at the same time and under the same environmental conditions as the descendants of the treated rats in the corresponding generation. As far as is known, no other experiment of this nature has been reported with controls so strictly comparable. Pearl (1917) fully recognized the importance of using controls comparable genetically, as well as otherwise, and planned to use brothers and sisters of his treated fowl as controls, but these plans failed to materialize. Stockard and Papanicolaou (1918) used guinea-pigs from the same stocks and blood lines and even used the same animals before treatment as a basis of estimating the effects of the alcohol treatment, but in this case the age factor is not equal in the two sets, and no comparable data on growth could be obtained. Nice (1912) and Arlitt (1919) merely used animals from the same dealers, as controls. Nice (1913) used mice in the fourth inbred generation from a single pair; Nice (1917) took all his mice from one strain which had been inbred for four generations after its start as the result of crossing two entirely distinct strains.

The alcohol was administered by the inhalation method. The treatment for both males and females started at the age of twenty-eight days, when they were weaned; to the males it was given daily throughout life;

to the females it was given daily with the exception of the twenty-eight days of nursing after the birth of each litter. The amount of alcohol inhaled was measured by the reactions of the rats. After the first days, when the treatment was lighter, they were left in the tanks until they were fully anesthetized, that is, limp and motionless.

The rats were raised on a diet of bread and fresh milk every day and dog bread especially prepared for rodents was kept in the cages at all times except during the behavior tests. Since all of these rats were used in behavior tests which involved reduction in the ration, the curves are lower at certain ages than they would normally have been. In all but a few cases the rats were weighed once a week on the same day of the week, starting with the first weighing day after they were weaned and continuing through the life of the animal. In a few cases at the end of the experiment, when war conditions made it impossible to make the weighings more often, they were made once a month. All available records have been used. From these records the growth curves were plotted on large sheets at the scale of twenty grams and ten days to the inch. Being weighed on the same day of each week the rats were at different ages; this necessitated a correction in order to summarize the data. The method employed was to interpolate the values at the desired ages by reading the weight value of the intersection of the age line with the straight line connecting the actual weighings plotted on either side. The possible effect of a personal element was eliminated in this way. In a preliminary study of these data an attempt was made to estimate corrections for minor irregularities in these curves and to take into consideration all special circumstances that might appear to influence the weights as recorded. This method of estimating the readings from the growth curves is obviously too open to criticism to be employed finally, but it may be noted that the results indicated by this method of reading the curves fully agreed with those here presented.

The main conclusions must be based on the males, since most of the females were bred and their pregnancies made a serious difficulty in reading the growth curves after maturity. No formulae could be used to correct for these pregnancy peaks, since the weighings were not made in any definite relation to the birth of the litters, so that the only way to include pregnant females without including the developing foetuses seemed to be to make an estimation of the probable normal weight based on the rest of the curve in comparison with the curves of the rest of the females in the same litter. Since there were frequently pregnancies in the other females in the litter, this estimation of normal weights for the females is far from reliable. Another complication lies in the fact that the treated

TABLE 1

Averages of the weight per male rat in grams for the different strains separately and together in each of the four generations.

A. Treated males

STRAIN		40 DAYS		60 DAYS		90 DAYS		120 DAYS		150 DAYS		180 DAYS	
		Means	No.	Means	No.	Means	No.	Means	No.	Means	No.	Means	No.
A	Tests	71.24	33	115.24	33	153.35	28	193.04	23	215.00	21	235.55	18
	Controls	73.29	27	130.00	27	179.08	24	231.28	21	256.60	20	287.93	15
B	Tests	75.28	7	126.37	8	149.62	8	197.33	6	277.75	4	232.00	2
	Controls	72.00	10	133.54	11	179.55	9	248 66	6	257.00	4	256.50	2
C	Tests	67.95	20	113.80	20	142.56	16	192.06	15	213.45	11	231.77	9
	Controls	71.57	19	124.73	19	172.78	14	226.46	13	250.25	12	264.40	10
L	Tests	69.30	10	119.30	10	150 66	9	183.33	6	206.00	2	246.00	5
	Controls	76.25	8	132.37	8	172.75	8	212.33	6	239.33	3	260.60	5
All	Tests	70.43	70	116.66	71	149.63	61	192.10	50	215.39	38	235.88	34
	Controls	72.95	64	129.35	65	176 63	55	229.71	46	257.97	39	274.34	32

B. Untreated males from treated parents

A	Tests	88.75	4	100.75	4	160.50	4	246.00	4	290.50	4	314.00	4
	Controls	59.10	10	75.00	10	111.80	10	200.25	8	247.50	8	276.00	8
B	Tests	75.50	6	93.66	6	117.16	6	181.83	6	212.16	6	218 33	6
	Controls	65.60	5	88.40	5	115.00	5	173.20	5	200.20	5	203.60	5
C	Tests	86.00	4	99.50	4	137.20	4	210.25	4	253.00	4	274.00	4
	Controls	63.66	3	78.00	3	123.33	3	180.66	3	199.00	3	212.33	3
L	Tests	62.25	12	93.41	12	117.91	12	180.08	12	212.00	12	233.00	11
	Controls	67.36	11	92.18	11	123.00	11	173.45	11	214.45	11	235.09	11
All	Tests	73.03	26	95.53	26	127.26	26	195.26	26	230.42	26	249.00	25
	Controls	63.82	29	84.13	29	117.79	29	182.14	27	219.88	27	238.85	27

C. Untreated males from untreated parents and treated grandparents

A	Tests	91.25	4	122.75	4	156.50	4	235.25	4	263.25	4	240.25	4
	Controls	95.66	3	126.66	3	197.66	3	270.00	3	307.66	3	315.33	3
C	Tests	66.00	3	95.66	3	128.66	3	193.66	3	227.66	3	226.33	3
	Controls	72.00	3	75.66	3	83.33	3	153.00	3	183.66	3	189.33	3
L	Tests	65.33	3	111.25	4	108.75	4	146.50	4	187.75	4	237.75	4
	Controls	61.00	1	91.25	4	104.00	4	111.00	4	151.50	4	197.00	4
All	Tests	75.90	10	111.18	11	131.54	11	191.63	11	226.09	11	235.54	11
	Controls	80.57	7	97.20	10	125.90	10	171.30	10	208.00	10	230.20	10

TABLE 1 (continued)

D. Treated males from treated parents

STRAIN		40 DAYS		60 DAYS		90 DAYS		120 DAYS		150 DAYS		180 DAYS	
		Means	No.	Means	No.	Means	No.	Means	No.	Means	No.	Means	No.
C	Tests	84.33	9	132.55	9	163.75	8	203.50	8	237.36	6	278.75	4
	Controls	81.10	10	136.00	10	161.60	10	202.88	9	234.88	9	255.37	8
L	Tests	83.16	6	121.16	6	170.16	6	190.83	6	219.33	6	226.00	6
	Controls	83.00	6	124.83	6	174.33	6	215.80	5	237.40	5	262.20	5
All	Tests	83.86	15	128.00	15	166.50	14	198 07	14	228 33	12	245.70	10
	Controls	81.81	16	131.81	16	166.37	16	207.50	14	236.50	14	258 00	13

females were less often pregnant than the controls; they produced less than half as many litters, while the offspring of the treated females produced nearly half as many again as their controls (MACDOWELL 1921). Supposing the larger the number of pregnancies the greater the inaccuracy, the weights estimated for the pregnant females among the controls would be more inaccurate than the tests in one generation and those for the tests would be more inaccurate than the controls in the other. The only justification for presenting the data for the females at all is that up to maturity they are as accurate as the males and taken altogether they form the only available material for comparison with the males.

The ages chosen for study are: forty days, sixty days, ninety days, one hundred and twenty days, one hundred and fifty days, and one hundred and eighty days. Thirty days would be the logical age to start with, but in many cases the first weighing was not made till after this age. At this time rats are growing very fast; to extend the curve backwards even a few days at this time introduces a large amount of inaccuracy due both to the small size of the weights and to the steepness of the curves as well as to the introduction of personal opinion. Every rat was weighed before forty days so the weight at this age could be interpolated without the chance of personal bias. Many rats were carried considerably beyond one hundred and eighty days, but the number of rats was much smaller after this age and the growth, aside from the deposition of fat, was practically completed by this time.

RESULTS

Summaries of the data are presented in the form of averages, for the males and females separately, for the test and control rats in each of the four strains used and for all the strains together, for each of the six ages

TABLE 2

Averages of the weight per female rat in grams for the different strains separately and together in each of the four generations.

STRAIN		40 DAYS		60 DAYS		90 DAYS		120 DAYS		150 DAYS		180 DAYS	
		Means	No.	Means	No.	Means	No.	Means	No.	Means	No.	Means	No.
		A. Treated females											
A	Tests	65.53	26	105.92	26	125.50	20	156.37	16	172.46	15	179.30	13
	Controls	69.50	26	112.46	26	134.08	23	169.72	18	184.70	17	195.76	17
B	Tests	65.00	5	103.14	7	122.14	7	160.66	6	166.66	3	175.50	2
	Controls	66.80	5	107.00	7	143.00	7	182.00	5	180.50	2	210.00	2
C	Tests	63.65	23	98.95	23	124.19	21	157.53	13	173.83	12	190.27	11
	Controls	65.09	22	108.86	22	138.00	19	182.38	13	188.00	11	205.72	11
L	Tests	64.27	11	105.54	11	117.87	8	145.71	7	157.25	4	182.00	2
	Controls	68.30	10	112.50	10	131.10	10	165.33	6	174.33	3	194.50	2
All	Tests	64.61	65	103.18	67	123.50	56	155.57	42	170.64	34	183.53	28
	Controls	67.55	63	110.66	65	135.89	59	174.47	42	184.60	33	200.00	32
		B. Untreated females from treated parents											
A	Tests	75.20	5	77.00	5	112.20	5	173.00	5	200.00	5	215.80	5
	Controls	63.50	4	78.75	4	107.00	4	161.50	4	192.25	4	208.50	4
B	Tests	71.33	6	81.33	6	101.33	6	146.16	6	167.16	6	175.66	6
	Controls	68.50	4	85.50	4	97.50	4	145.50	4	168.50	4	173.50	4
C	Tests	74.00	2	83.00	2	110.50	2	156.00	2	172.00	2	184.00	2
	Controls	58.33	3	67.66	3	91.00	3	152.00	3	179.00	3	188.00	3
L	Tests	64.22	9	89.55	9	96 33	9	154.55	9	182.77	9	190.44	9
	Controls	64.00	14	81.00	14	100.57	14	154.30	13	171.92	13	184.69	13
All	Tests	69.54	22	83.86	22	102.59	22	156.59	22	181.45	22	191.59	22
	Controls	63.96	25	79.76	25	99.96	25	153.75	24	175.62	24	187.20	24
		C. Untreated females from untreated parents and treated grandparents											
A	Tests	84.00	1	91.00	1	120.00	1	176.00	1	201.00	1	195.00	1
	Controls	86.00	3	120.00	3	138.60	3	182.30	3	216.60	3	220.00	3
C	Tests	60.00	2	79.50	2	100.00	2	139.00	2	167.00	2	....	...
	Controls	76.00	2	72.50	2	76.50	2	140.50	2	180.00	2	184.00	2
L	Tests	62.85	7	99.00	7	100.14	7	126.57	7	149.43	7	182.66	6
	Controls	57.00	2	81.70	7	93.20	7	102.20	.7	135.20	7	171.50	7
All	Tests	64.40	10	94.30	10	102.10	10	134.00	10	158.10	10	184.42	7
	Controls	74.85	7	85.25	12	101.88	12	128.66	12	163.08	12	182.63	11

TABLE 2 (continued)

D. Treated females from treated parents

STRAIN		40 DAYS		60 DAYS		90 DAYS		120 DAYS		150 DAYS		180 DAYS	
		Means	No.	Means	No.	Means	No.	Means	No.	Means	No.	Means	No.
C	Tests	74.00	9	110.11	9	138.33	6	166.60	5	183.25	4	201.50	4
	Controls	68.00	11	106.75	12	125.25	12	162.88	9	176.66	9	184.44	9
L	Tests	73.14	7	103.42	7	129.28	7	153.16	6	160.66	3	170.66	3
	Controls	70.71	7	100.85	7	134.42	7	158.83	6	175.25	4	179.33	3
All	Tests	73.62	16	107.18	16	133.46	13	159.27	11	173.57	7	188.28	7
	Controls	69.05	18	104.57	19	128.63	19	161.26	15	176.23	13	183.16	12

TABLE 3

Differences between the average weights of the tests and controls when the strains in each generation are taken together; when the average of the controls is greater, the difference is given a plus sign (+).

AGE	TREATED RATS		PARENTS TREATED		GRANDPARENTS TREATED		TREATED FROM TREATED PARENTS	
	Differences	Diff. / P. E.	Differences	Diff. / P. E.	Differences	Diff. / P. E.	Differences	Diff. / P. E.
	Males		Males		Males		Males	
40	+ 2.52±1.40	1.8	− 9.21±2.37	3.8	+ 4.67± 5.08	0.9	− 2.05±2.37	0.7
60	+12.69±2.16	5.9	−11.40±2.56	4.4	−13.98± 5.36	2.6	+ 3.81±3.81	1.0
90	+27.00± .316	8.5	− 9.47+3.64	2.6	− 5.64±11.64	0.5	− 0.13±4.96	0.0
120	+37.61±4.07	9.2	−13.12±5.13	2.5	−20.33±16.66	1.2	+ 9.43±6.01	1.5
150	+42.58±4.81	8.8	−10 54±6.24	1.6	−18 09±16.20	1.1	+ 8.17±6.48	1.2
180	+38.46±5.11	7 5	−10.15±7.61	1.3	− 5 34±13 52	0.4	+12.30±7.44	1.6
	Females		Females		Females		Females	
40	+ 2.94±1.16	2.5	− 5.58±2.36	2.3	+10.45±4.25	2.4	− 4 57±1.99	2.3
60	+ 7.48±1.63	4.6	− 4.10±2.35	1.7	− 9.05±3.21	2.8	− 2.61±3.04	0.8
90	+12.39±4.67	2.6	− 2.63±2.80	0.9	− 0.22±5.10	0.0	− 4.83±3.30	1.4
120	+18.90±2.97	6.3	− 2.84±2.94	0.9	− 5.34±7.87	0.6	+ 1.99±3.62	0.5
150	+13.96±3.26	4.3	− 5.83±3.38	1.7	+ 4 98±8.20	0.6	+ 2.66±4.35	0.6
180	+16 47±3 53	4 6	− 4 39±3.41	1 3	− 1.79±4 95	0.3	− 5.12±5.64	0.9

studied (tables 1 and 2). The number of rats included in each average is indicated in the column immediately following it; just below each test average is placed the corresponding control average. Four generations are given: (1) treated rats (the first ones treated); (2) their children (untreated from treated parents); (3) their untreated grandchildren (untreated from untreated parents and treated grandparents); (4) children of treated rats that were themselves treated (treated from treated parents). All the averages are shown graphically in figures 1 and 3; the broken lines represent the tests (treated rats or their descendants), the solid lines the controls in the respective generations and strains. The ages in days are shown on the base lines and the ordinates represent the weights in grams.

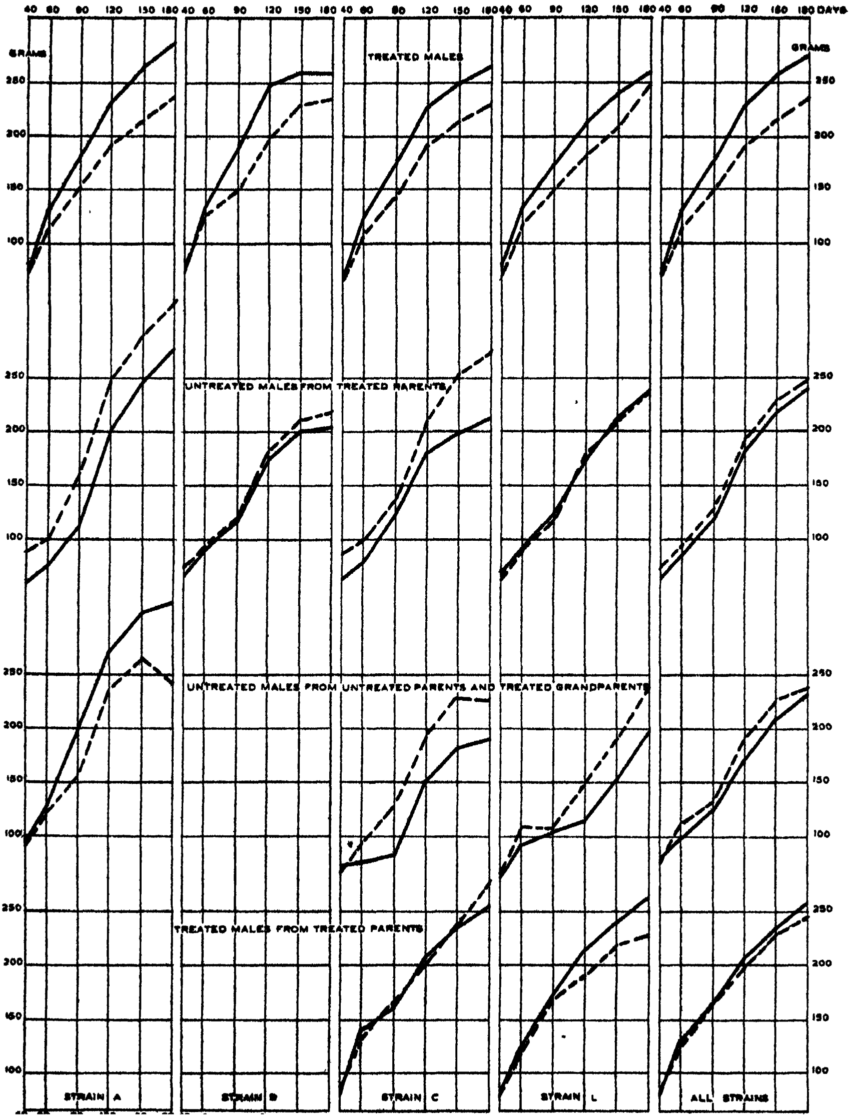

FIGURE 1.—Growth curves of the test and control males, plotted from the data given in table 1. The broken lines represent the tests (treated rats or descendants of treated rats), the solid lines the controls. The curves in the same vertical column belong to the same strain; the column at the right includes all strains; the horizontal rows of curves represent the different generations.

The curves are arranged in the chart so that the different generations of one strain lie in the same vertical column; in the column at the right the averages (primary averages, not averages of strain averages) for all strains together are shown. All the curves, those for the test rats as well as for the controls, are lower at ninety days than they normally should be, due to the light feeding that was administered in connection with the maze training that was being given to both groups at this time.

Treated rats

A part of the weight data from treated males has already been presented (MACDOWELL and VICARI 1917) in a preliminary report; the addition of more data in the present report does not change the earlier conclusions. The treated males grow less rapidly than the controls. This is true whether the strains are considered separately or together. At forty days the weights are very nearly equal, although in strains C and L controls are already higher than the tests. The data available for thirty days indicate that the weights for this time were alike for the two groups. This is the result of making a purely random division of the males and females in each litter into two groups, those to be treated and those to be used as controls. Table 3 gives the differences between the averages for the treated rats and their controls when all strains are taken together. With the exception of the first comparison (at 40 days) all ages give differences that are unquestionably significant; the smallest of these, occurring at 60 days, is 5.9 times the probable error, three times the probable error being the recognized criterion of statistical significance. The frequency distributions of the individual weights of the males, all strains taken together, are given in figure 2. There is a pair of frequency polygons for each age. Beginning with forty days in the lower left-hand corner, the successive curves give the distributions for 60, 90, 120, 150, and 180 days. With the exception of the pair for 40 days, these curves are plotted on a percentage basis; the small amount of variation in the weights at 40 days has led to the use of classes half as wide in classifying these data. In order to compensate for this and make the actual area included by the curves equal to the others they have been plotted on the basis of two hundred instead of percent. The base lines are scales of weight in grams and all the curves are drawn on the same continuous scale; the numbers given are the lower limits of the respective classes. The vertical scales are the proportions in each weight class of the total number of individuals. Although there is very little difference between the distributions of the treated rats and their controls at forty days, a tendency is already suggested for the treated rats to be lighter; there is a smaller proportion of the treated rats in classes of 80, 90 and 100 grams and a larger proportion of them in classes of 60 and 70 grams. At sixty days this tendency is more marked; the proportion of treated rats in classes of 80, 100 and 120 grams is greater than the controls in these classes, but there is a higher proportion of controls in classes of 140 and 160 grams.

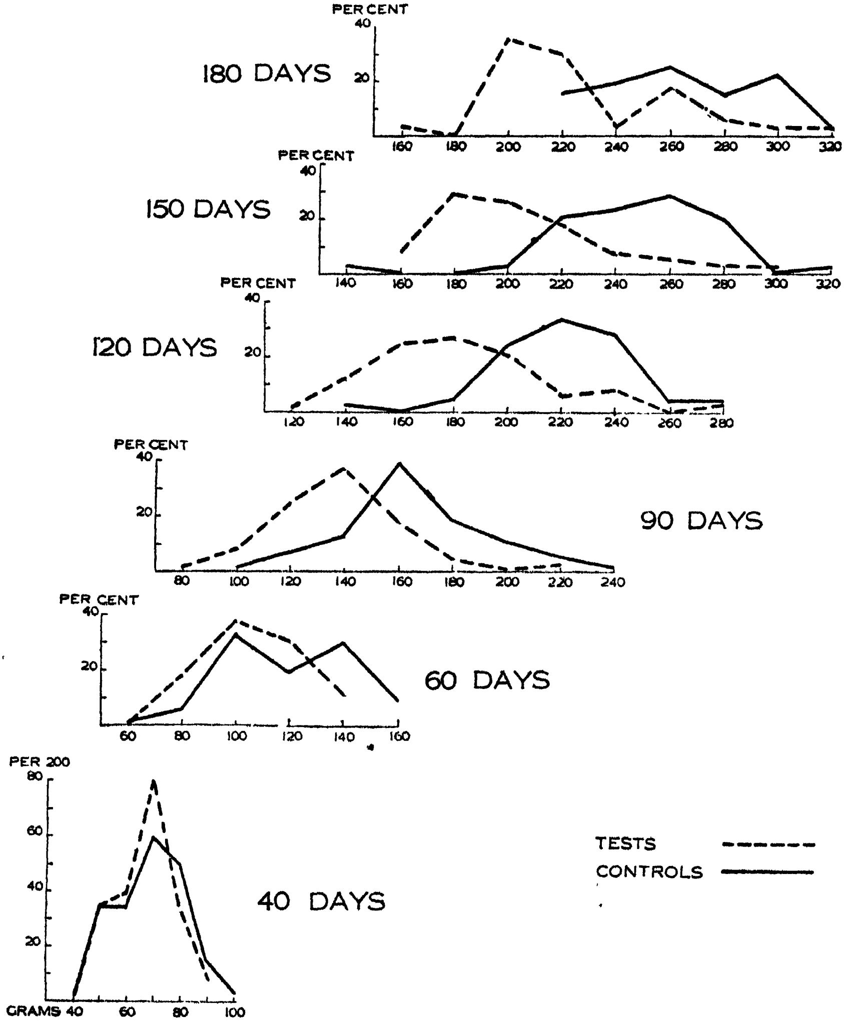

FIGURE 2.—Frequency distributions of the weights of the treated males at the different ages. The broken lines are the tests and the solid lines the controls; all curves are drawn on the same scale of weights, but the frequency scale is repeated for each pair of curves. All frequencies are in percents but those for forty days, which are given per 200, as the class width for 40 days is half the size of the others.

At 90 days the curve of the treated rats has drawn well away from that of the controls so that the two modes fall in separate classes. The curves cross between the classes of 140 and 160 grams; below the point of crossing there is a higher proportion of treated rats in each class; above the point of crossing there is a higher proportion of controls. By 120 days the modes of the two distributions are two classes apart, with greater differences on

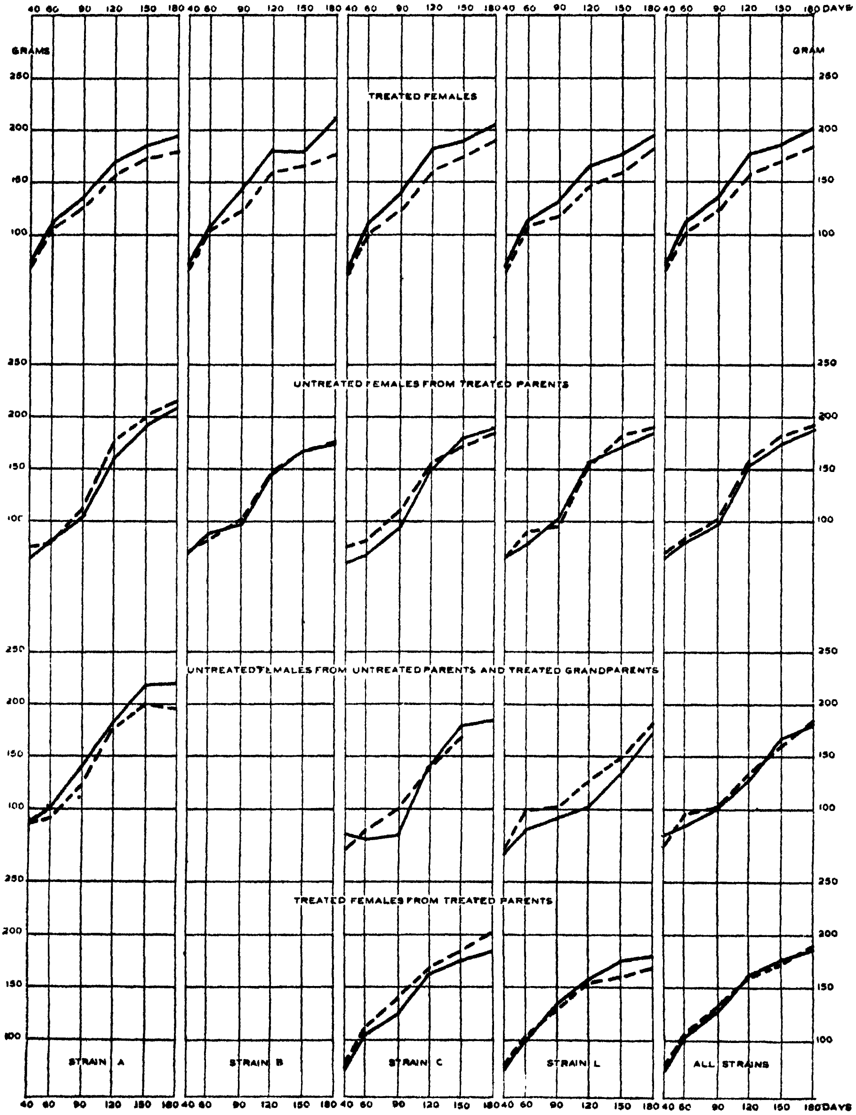

FIGURE 3.—Growth curves of the test and control females plotted from the data given in table 2. The broken lines represent the tests, the solid lines the controls.

either side of the crossing point. By 150 days the modes are five classes apart. The numbers are smaller at 180 days and this shows in the irregularity of the curves. There is only a little advance in weight over the 150-day distributions as a whole, but the concentration of the treated rats below the mass of controls is clearly more marked. During this progressive separation of the two distributions there have remained a few treated rats as heavy as the heaviest controls, but there can be no doubt that a real lowering of the treated group as a whole has occurred. The results given by the averages are not due to some erratic distribution of a few individuals but to the general movement of the population as a whole. Turning to figure 3, which gives the results for the females corresponding to the males in figure 1, the averages for treated females are found in the first row of curves. These curves are plotted on the same scale as those for the males and may be compared directly; the ruling of the background is also the same for both figures, and the strains given in the same order. The first thing to attract attention is that the females do not grow so fast as the males and by 180 days they are considerably lighter,—a well known fact but one sometimes not taken into account. Compared with their untreated sisters (controls) the treated females average lower at each age. At forty days the difference has already appeared in each strain, although like the males, they were probably equal at thirty days. For the later ages the absolute differences between the averages of the treated and control females are not as great as they are for the males. Table 3 shows that, when all strains are taken together, the differences are more than four times their probable errors for the ages of 60, 120, 150 and 180 days, and over twice their probable errors for the ages of 40 and 90 days.

The general conclusion may be stated: The treatment of white rats with maximum doses of alcohol by the inhalation method tends to reduce the rate of growth of the treated animals as compared with their untreated sibs raised at the same time and under the same conditions. This appears in the averages and the distributions but it does not affect all individuals alike, since some remain as heavy as the heaviest controls. In spite of the smaller absolute differences and the unreliability occasioned by their pregnancies, the females give the same general results as the males.

Untreated rats from treated parents

The second row of curves in figures 1 and 3 shows the averages of the weights of the offspring resulting from matings between the treated males and treated females (sisters by brothers) and between the controls (sisters by brothers).

In this and the following generations all the rats were trained in a multiple-choice apparatus as well as in the maze; this occasioned a continuation of the light feeding for a longer time, so that the weights for 120 days as well as for 90 days are lower than they would be normally. The males from treated parents in strains A and C have higher averages than the controls at all ages; the same difference is present in strain B, although not so strongly marked. In strain L there is no difference between the curves for the tests and controls. When the four strains are combined parallel curves result, with the tests heavier throughout. Table 3 indicates that the differences are probably statistically significant for the first three ages (being 3.8, 4.4, 2.6 and 2.5 times their respective probable errors), but for the last two ages they are not significant. The females in this generation show less consistent results, but the tendency for the tests to be heavier than the controls appears in strains A and C; and when all the strains are put together, the test and control curves are parallel, with the tests heavier than the controls at all ages. The differences are so small, however, that, in comparison with their probable errors (see table 3) they can not be considered statistically significant. In spite of this, the direction of the differences and their consistency in the different ages support the conclusion warranted by the results from the males, that the offspring of the treated rats tend to be heavier than the offspring from the controls.

Untreated rats from untreated parents and treated grandparents

This generation was produced by inbreeding rats in the preceding generation, the tests by their own sibs and the controls by their own sibs. Strain B was not carried to this generation. The numbers raised, as indicated in table 1, are very small. For the males in strains C and L the test averages are heavier than the controls; strain A, on the other hand, shows the controls heavier than the tests (see third row of curves in figures 1 and 3). The females also show the controls heavier in strain A and the tests in strain L, but in strain C there is no consistent difference between the tests and controls. When all strains are put together the averages show the test males higher than the controls and practically no difference at all between the test and control females. At 60 days the differences for both males and females are in favor of the tests and they are probably statistically significant, being 2.6 and 2.8 times their probable errors; the only other difference that approaches three times its probable error is for the females at 40 days when the control average is higher, the difference being 2.4 times its probable error.

No definite conclusion can be drawn from such results. The numbers are too small, in the absence of consistency in the different strains and the sexes, to prove anything; but, as far as any general difference between the tests and controls is indicated, it appears to be in favor of the tests.

Treated rats from treated parents

Some of the rats in strains C and L produced by the first treated generation were themselves given the treatment. This treatment was started when the animals were weaned; it was given full strength at once, that is, without any preliminary lighter treatment, the rats were immediately left in the fumes each day until they became dead drunk. Weights are given for thirty-one animals so treated and thirty-four controls. As far as their breeding is concerned these rats are comparable with the untreated rats from treated parents whose weights are represented in the second row of curves in figures 1 and 3. The curves (the bottom row in figures 1 and 3) show that the test males in strain C were almost exactly equal to the controls; the test females in this strain were slightly heavier than the controls. In strain L the test males were slightly lower than the controls in the later ages, but the test females barely suggest a similar lowering of the tests at the ages of 150 and 180 days. With the strains taken together, there appears to be no general difference in either sex between the tests and controls; none of the differences found at individual ages is significant.

DISCUSSION

Reviewing the results in all the different generations, the following statements may be made: Treating rats with strong doses of alcohol fumes tends definitely to reduce the rate of their growth, but the untreated offspring of such treated rats tend to be heavier than the controls. Although no generalization can be made for the grandchildren of the treated rats, the small number of data at hand suggest that they too tend to be heavier than the controls. While the treatment of one generation reduces the rate of growth, the treatment of rats from treated parents seems to have no effect at all upon the rate of their growth.

Taken by themselves such results appear to be inconsistent and unsatisfactory, but taken in connection with the results of the study of the fertility of these same rats and in the light of PEARL's (1917) suggestion of the selective effect of alcohol, they are not at all surprising. It has been shown (MACDOWELL 1921) that the treatment of these rats reduced the expected number of litters. Compared with the untreated sibs, the treated rats failed to produce over 64.8 percent of the litters expected, but the offspring they did raise in turn gave birth to 33.3 percent *more* litters than the controls in the same time. These are definite results and they give a clear indication of how the data are to be interpreted. It is only necessary to suppose a certain amount of correlation between weight and the ability to produce litters, to understand how the alcohol could reduce the weights in one generation and cause an increase over the controls in the next generation. If the alcohol should prevent certain females from having any litters and these females were the ones carrying genes for lighter weight as well as poorer reproductive capacity, the offspring that were produced would be a selected lot as compared with the controls, and they would bear fewer genes for infertility and fewer for light weight, supposing these genes are different. The generations following should still show the superiority of the descendants of the treated rats. The fertility data show this clearly in spite of the small numbers, but the weight data are too variable to give clear results. For the males there did appear to be a slight tendency for the tests to average higher.

If the application of alcohol to one generation reduces the rate of growth, why does not the treatment of the offspring as well cause a reduction in weight as great or greater? The facts are clear that such a reduction in the weights of treated offspring is not found, and equally clear that the treatment of the first generation has the immediate effect of reducing the rate of growth in most rats. The lack of modification found when the treated rats came from treated parents can be explained by supposing them to be a selected lot superior genetically to the controls, so the treatment may reduce their potential weight and yet leave them no lighter than the controls. This is the result to be expected when the immediate somatic tendency of the alcohol to reduce weight, and its indirect genetic tendency to cause an increase in weight are both working upon the same animals.

RELATED INVESTIGATIONS

In spite of the vast literature on alcoholism, there are very few papers giving experimental results of the effect of alcohol on the growth of animals. For purposes of comparison certain of these will be cited. ARLITT (1919) reported weighing white rats that were treated with alcohol, as well as their descendants. She concluded (p. 10) that,

> "In all cases alcohol causes a marked retardation in the rate of growth as judged by the disparity in body weight of alcoholics as compared with normals of the same age. Parental alcoholism has a more marked effect on the rate

of growth than is present when the drug is administered to the animals themselves, as is obvious from analysis of the growth curves shown in figure II."

In the third and fourth generations this retardation in weight is present to a slightly less degree. "The defective body weight acquired by alcoholized rats is also inherited" (p. 12). In spite of this sweeping claim to have proved the inheritance of an acquired character, no critical estimation of any of these conclusions is possible. The data are presented in the form of curves, with no indication of the number of animals involved, with no apparent recognition of the sexual difference in weight, and with no information as to the genetic or environmental qualifications of the controls. A careful study reveals the fact that the same control data are used for comparison with each of the four generations, although this is obscured by the use of three different scales in plotting the four generations.

STOCKARD and PAPANICOLAOU (1917) give data from guinea-pigs on the birth weights and weights at the end of the first and third months. The summaries would seem to indicate that normal offspring weigh more, and shortly after birth, grow more rapidly than the young of alcoholic guinea-pigs. A complicated relationship is shown between the birth weights and growth and the number in the litter. Since small litters tend to have heavier offspring, and since the test animals tend to have smaller litters, the apparent effects of alcohol are minimized. There is no separation of the data on the basis of the number of generations from treatment.

NICE (1912) reported that alcohol fed to adult white mice had a fattening effect upon them; they grew heavier than the controls. The treated offspring of these treated adults also surpassed the controls but the untreated offspring from the same parents grew faster still. Unfortunately the controls were probably not genetically comparable; all the mice first treated were secured "from a dealer and guaranteed not to be brothers and sisters," and when those originally chosen as controls proved to be unsatisfactory breeders, others were obtained "from the same source as the first lot of mice so as to serve as controls for comparison with the second generation of other lines." In view of these facts, the comparisons between the treated rats and their controls have very uncertain significance. On the other hand, the comparison of treated offspring with untreated offspring from the same parents is not open to the same uncertainty, since the probability of genetic similarity between the two sets is greatly increased. This comparison shows that, parentage being equal, mice treated with alcohol from the age of three weeks are lighter than their untreated brothers and sisters. This is the one case in which treatment was begun before the animals were adult; it is accordingly comparable with the experiments herein reported and gives the same general result as far as it goes.

Later, NICE (1917) repeated these experiments: "the white mice given alcohol by the inhalation method gave much the same results as those that received it in their food in my former experiments" (p. 606). It should be noted however that the whole stock of mice used in this experiment came from the same known strain so that the controls were more comparable than in the first experiment, and instead of growing more rapidly, the first mice treated (started when 11 weeks old) gained weight less rapidly than the controls. The offspring of the treated mice grew faster than the controls, as was the case with the rats in the present experiment, but their superiority over the controls seemed to be maintained whether further treatment was given or not. In connection with a study of the effects of alcohol upon the acitivity of mice, NICE (1913) gave further data on weight. Records of four treated mice and four controls are given. Qualitatively these controls seem to be more satisfactory than any others that have been presented for the study of the immediate effects of alcohol; they were all of the same sex and closely related; however the dosage was very light, consisting of 3 cc of 35 percent alcohol given with the food every other day, and 35 percent alcohol instead of water to drink, and the treatment did not begin till the mice were practically grown at 8 weeks. In ten of the eleven weighings that were made the control averages were higher, but at the beginning of the experiment the controls averaged 1.16 grams higher than the treated mice, and this difference is not exceeded at any other age but the last (36 weeks) when two of the four treated mice had died. If this has no other significance, it shows that the supposition that alcohol has a general tendency to fatten does not hold.

In comparing the growth curves of fowl treated with ethyl and methyl alcohol with those of the untreated birds, PEARL (1917) found that no "profound or far-reaching effect upon the general metabolic processes of these birds has as yet been produced by the treatment" (p. 175). This conclusion is based on 15 monthly weighings of 14 treated birds (10 hens and 4 cocks) and four weighings of the controls (supposedly 39 hens). The treated hens are heavier than the controls between November and January of the first year and between September and January of the second year. From January to June there is a great reduction in the weight of the treated hens, but since there are no control data for this period it cannot be proven that this drop is due to the treatment. However PEARL considers that this is probably the first significant effect of the administration of the alcohol. A full series of weights of the offspring of treated birds and controls is given from hatching to about three hundred days. The offspring of the treated birds tend to grow heavier than the controls, although this difference does not appear for three or four months after hatching.

SUMMARY

Data presented in this paper would seem to warrant the following conclusions:

1. Treatment of white rats with the fumes of alcohol from the time of weaning to maturity reduces the rate of growth as compared with that of their untreated brothers and sisters.
2. The untreated male offspring of treated rats are heavier on the average than the controls in this generation.
3. There is some indication of a slight tendency for the male grandchildren of the treated rats to be heavier than the controls in their generation.
4. The male offspring of treated rats that are themselves treated show no differences in weight from their controls.
5. In all cases the females give less reliable results than the males on account of the pregnancies, but in so far as they show anything they support the results based on the males alone.
6. These results may be interpreted by supposing that the alcohol treatment has two opposing tendencies, namely, (1) a tendency to retard the normal rate of growth, and (2) a tendency to prevent the reproduction of those females bearing weaker-growth genes and so to raise the weight average in the next generation.

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THE CORRELATION BETWEEN THE EGG PRODUCTION OF THE VARIOUS PERIODS OF THE YEAR IN THE RHODE ISLAND RED BREED OF DOMESTIC FOWL

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INTRODUCTION

This paper has for its purpose the consideration of the correlation between the egg records of various periods of the year in the Rhode Island Red breed of domestic fowl. Such correlations have already been determined and are in part published for the White Leghorn breed (HARRIS, BLAKESLEE and WARNER 1917; HARRIS, BLAKESLEE, WARNER and KIRKPATRICK 1917; HARRIS, BLAKESLEE and KIRKPATRICK 1917, 1918). In the discussion of the present investigation, we shall assume a knowledge of the results of these earlier studies, particularly that on the correlation between the records of various periods in the White Leghorn (HARRIS, BLAKESLEE and KIRKPATRICK 1918).

In addition to the general biological importance of the knowledge of the interrelationship between the physiological activities of the organisms of a population at various periods, such as will result from the determination of these correlations, the investigations have a potential practical significance. The existence of a correlation between the records of the individual months and the record of the year as a whole is the basis upon which the possibility of the prediction of the record of groups of birds for longer periods from the measured record of a short period of time must rest. In the case of the White Leghorn fowl, such prediction has been shown to be possible with a high degree of accuracy[1] (HARRIS, KIRKPATRICK and BLAKESLEE 1921; HARRIS, KIRKPATRICK, BLAKESLEE, WARNER and CARD 1921). It is, therefore, important to determine whether correlations of a similar magnitude also exist in breeds other than the White Leghorn. The investigation of the correlation existing in an individual flock is also a matter of considerable practical interest. Our earlier correlations, both those deduced from the Storrs series (HARRIS and others, loc. cit.) and from the Vineland series (HARRIS and LEWIS 1921) were based upon series of birds which were submitted by widely scattered breeders. There is a possibility of the correlations deduced in these cases being considered as due to the heterogeneity of materials thus assembled. This point can be settled only by determining correlations for individual flocks and comparing these with correlations deduced from contest samples drawn from many flocks. It is evident that if the methods of prediction of egg production are to be commercially applied, the practical use must be made in individual flocks. It is, therefore, economically essential to determine whether correlations are found in individual flocks similar to those which have been demonstrated in contest series.

It must not be inferred that we are oblivious to the many biological factors, extrinsic as well as intrinsic, upon which fecundity depends. Certain of these factors, such as time of maturity and broodiness, have been especially emphasized by one of us (GOODALE 1918 a, b, 1920). The great labor of calculation renders it impossible to treat all of the variables simultaneously. The analysis must, therefore, proceed step by step. Ultimately we hope to study in greater statistical detail the interrelationship of the various factors which for the moment must be left out of account.

The present paper on the Rhode Island Red breed is one of a series to be devoted to the more important breeds. A general comparison of results is reserved until the detailed presentation of the constants for each of the breeds is completed.

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[1] While it is possible to make predictions for groups of birds, which are highly accurate on the average, it must not be inferred that it is possible to apply these equations to the prediction of the production of specific individuals.

MATERIALS

The materials upon which this study is based are the egg records for the pullet year made by the Rhode Island Red flocks at the MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION for the laying years 1912-1913 to 1918-1919. Records made by birds that died or became pathological are omitted. The foundation stock, 1912-1913, came mainly, but not exclusively, from a single breeder. In 1915-1916, considerable new blood was added. During the period under consideration, breeding experiments dealing with egg production were going on, but aside from this fact, the flocks are a fair sample of what might be obtained from trap-nest records on any commercial poultry farm of similar size. Every care has been taken to keep the daily records accurately and to maintain all controllable environmental factors uniform.

In order to secure as many checks as possible on the trustworthiness of the constants it has seemed desirable to treat the materials in three groups. We have, therefore, determined the correlations for the 443 birds of 1915-1916, for the 442 birds of 1916-1917 and finally for all the birds of the seven years.

PRESENTATION AND ANALYSIS OF DATA

The correlation between the record of the individual months and annual production

The correlation between the production of the individual months of the year and the annual egg record for the series for the two individual years, 1915-1916 and 1916-1917 appear in table 1. The correlations for the whole period, 1912-1919, are given in the first section of table 2.

It is clear that the correlations are of a substantial magnitude and statistically significant in comparison with their probable errors throughout. They are thus in full general agreement with the results obtained from the White Leghorn birds exhibited at the INTERNATIONAL EGG LAYING CONTEST at Storrs, Connecticut.

The constants indicate, therefore, that the production of any individual month should furnish a reasonably satisfactory basis for the prediction of the annual egg production of the Rhode Island Red fowl just as it has been shown to be in the case of the White Leghorn fowl.

It is interesting to note from the difference column in table 1 that the differences between the correlation coefficients deducted for the two different years are in general small. Five of them are less than twice as large as their probable errors. In view of the fact that less than five

TABLE 1

Correlation between the egg production of twelve individual months and total annual egg production.

MONTHS	CORRELATIONS		DIFFERENCE (1916-1917) −(1915-1916)	$\frac{\text{DIFF.}}{E_{\text{diff.}}}$
	1915-1916	1916-1917		
November	.3245 ± .0287	.4357 ± .0255	+.1112 ± .0384	2.90
December	.4887 ± .0244	.6646 ± .0179	+.1759 ± .0302	5.82
January	.5753 ± .0214	.5910 ± .0209	+.0157 ± .0299	0.53
February	.5092 ± .0237	.5937 ± 0208	+.0845 ± .0315	2.68
March	.5020 ± .0240	.5763 ± .0214	+.0743 ± .0321	2.31
April	.5522 ± .0223	.4427 ± .0258	−.1095 ± .0341	3.21
May	.5255 ± .0232	4798 ± .0247	−.0457 ± .0353	1.29
June	.5395 ± .0227	.5460 ± .0225	+.0065 ± .0320	0 20
July	.5585 ± .0221	.5623 ± .0219	+.0038 ± .0311	0.12
August	.6021 ± .0204	.5522 ± .0223	−.0499 ± .0302	1.65
September	.5374 ± .0228	.6321 ± .0193	+.0947 ± .0299	3.17
October	3978 ± 0270	.4979 ± 0241	+.1001 ± .0362	2 77

TABLE 2

Comparison of correlation between the egg production of the twelve individual months and total annual egg production in the Rhode Island Red and the White Leghorn breeds.

MONTHS	CORRELATIONS		DIFFERENCE (R I.R)−(W.L.)	$\frac{\text{DIFF}}{E_{\text{diff.}}}$
	Rhode Island Red 1912 1913 to 1918-1919	Storrs White Leghorn 1911-1912 to 1916-1917		
November	.4678 ± 0130	4040 ± 0132	+ 0638 ± .0185	3.45
December	5991 ± 0106	5244 ± 0114	+.0747 ± .0156	4.79
January	6349 ± .0099	.5062 ± 0117	+.1287 ± .0153	8.41
February	.5720 ± .0112	5360 ± .0112	+.0360 ± .0158	2.28
March	.5553 ± .0115	.5769 ± 0105	−.0216 ± .0156	1 38
April	.4761 ± 0128	5469 ± .0110	− 0708 ± .0169	4.19
May	4401 ± 0134	.5904 ± 0102	− 1503 ± .0169	8 89
June	.5173 ± 0122	.6496 ± .0091	−.1323 ± .0152	8.70
July	.5361 ± .0118	.6711 ± 0086	−.1350 ± .0146	9.25
August	.5682 ± .0112	6700 ± .0087	−.1018 ± .0142	7.17
September	5940 ± .0107	.6526 ± 0090	−.0586 ± .0140	4.19
October	4742 ± 0129	5251 ± .0114	−.0509 ± 0172	2.96

hundred birds are involved in each series and that these birds have been subject to the differences in conditions of two years, the agreement may be considered excellent. This will be shown in another way later.

While a more detailed comparison between breeds is reserved until those other than the Rhode Island Red and the White Leghorn may be taken into account, it is interesting to compare in a wholly preliminary way the results (hitherto unpublished) obtained from the data for White Leghorn birds as observed at Storrs during the six years 1911 to 1917. These are laid beside the series of Rhode Island Red birds observed at Amherst during the period 1912 to 1919 in table 2. The differences are in general much larger than those between the two series of Rhode Island Red birds compared above, and suggest that the two breeds may differ in their correlations. This furnishes an additional reason for the carrying out of a series of comparative studies on this problem.

For purposes of prediction we must pass from measures of interrelationship in terms of correlation to those expressed in terms of regression equations.

TABLE 3

Regression equations for the prediction of annual total egg production from monthly egg records.

MONTHS		REGRESSION EQUATIONS		
		1915-1916	1916-1917	1912-1913 to 1918-1919
November	e_1	$E=116.853+2.174\ e_1$	$E=116.653+2.600\ e_1$	$E=118.050+2.794\ e_1$
December	e_2	$E=108.366+2.406\ e_2$	$E=95.301+3.274\ e_2$	$E=106.027+2.987\ e_2$
January	e_3	$E=97.114+2.695\ e_3$	$E=95.751+2.921\ e_3$	$E=95.609+3.104\ e_3$
February	e_4	$E=91.093+2.551\ e_4$	$E=89.727+3.342\ e_4$	$E=88.828+3.249\ e_4$
March	e_5	$E=66.974+3.079\ e_5$	$E=42.661+4.452\ e_5$	$E=58.396+3.880\ e_5$
April	e_6	$E=50.253+4.062\ e_6$	$E=53.619+4.046\ e_6$	$E=60.015+3.895\ e_6$
May	e_7	$E=61.579+3.832\ e_7$	$E=65.176+4.141\ e_7$	$E=73.194+3.579\ e_7$
June	e_8	$E=74.667+3.915\ e_8$	$E=76.134+5.048\ e_8$	$E=78.474+4.233\ e_8$
July	e_9	$E=84.414+3.920\ e_9$	$E=91.883+4.459\ e_9$	$E=90.035+3.903\ e_9$
August	e_{10}	$E=87.999+4.046\ e_{10}$	$E=100.595+3.597\ e_{10}$	$E=94.704+3.929\ e_{10}$
September	e_{11}	$E=101.996+3.076\ e_{11}$	$E=99.090+4.015\ e_{11}$	$E=101.666+3.754\ e_{11}$
October	e_{12}	$E=113.621+2.574\ e_{12}$	$E=115.121+3.644\ e_{12}$	$E=116.928+3.402\ e_{12}$

This is essential not merely for purposes of testing the possibilities of prediction but for verifying the validity of the correlation coefficient as applied to the particular data in hand, the validity of the product-moment method of determining closeness of interrelationship depending upon linearity of regression.

The linear regression equations for the two years 1915-1916 and 1916-1917 appear in table 3. These equations are represented graphically in diagrams 1 to 3.

On these diagrams the results for the two years are laid side by side. The figures show: (1) a reasonable agreement of the results obtained for the two different years; and (2) a reasonable agreement of the empirical annual mean production of birds laying various numbers of eggs in the individual months of the year with the fitted straight lines. Taking the series as a whole, the closeness of agreement, as judged by inspection, merely, would seem to be very comparable with that obtained from the two-years production of White Leghorn birds, submitted by a large number of breeders in the INTERNATIONAL EGG LAYING CONTEST at Storrs (HARRIS, BLAKESLEE and KIRKPATRICK 1918, figures 2–5). While it can not be asserted that regression is strictly linear, these results indicate

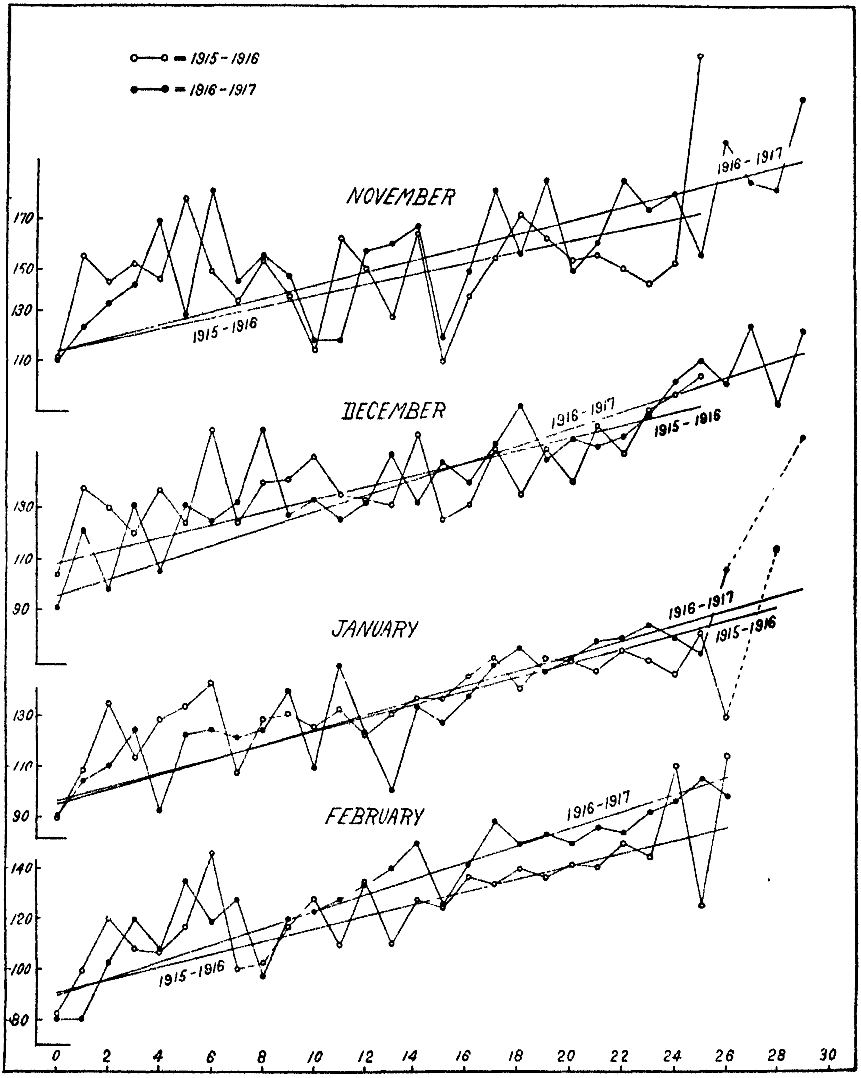

DIAGRAM 1.—Regression of annual production on monthly production.

unmistakably that prediction of the annual record of the Rhode Island Red fowl might be made with a relatively high degree of precision from the recorded performance of any individual month, just as has been shown to be possible in the case of the White Leghorn breed (Harris, Kirkpatrick, Blakeslee, Warner and Card 1921).

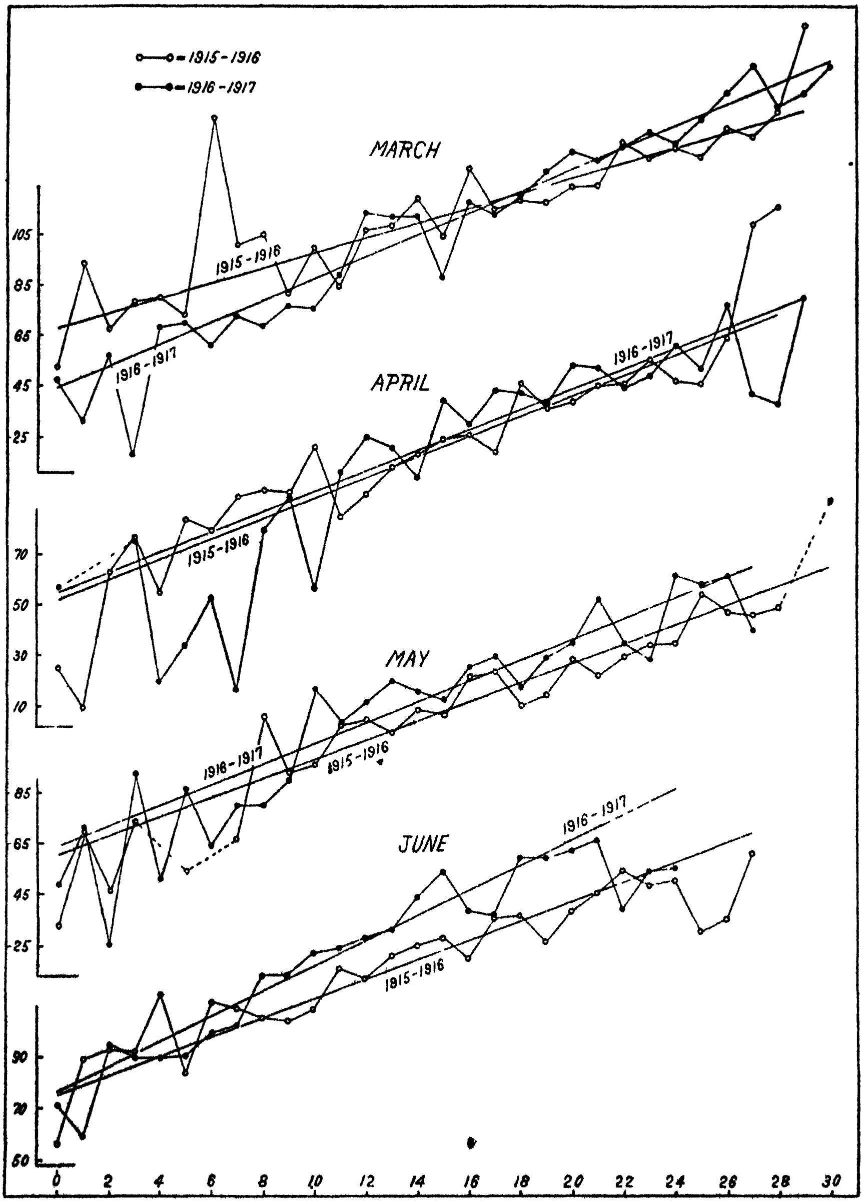

Diagram 2.—Regression of annual production on monthly production.

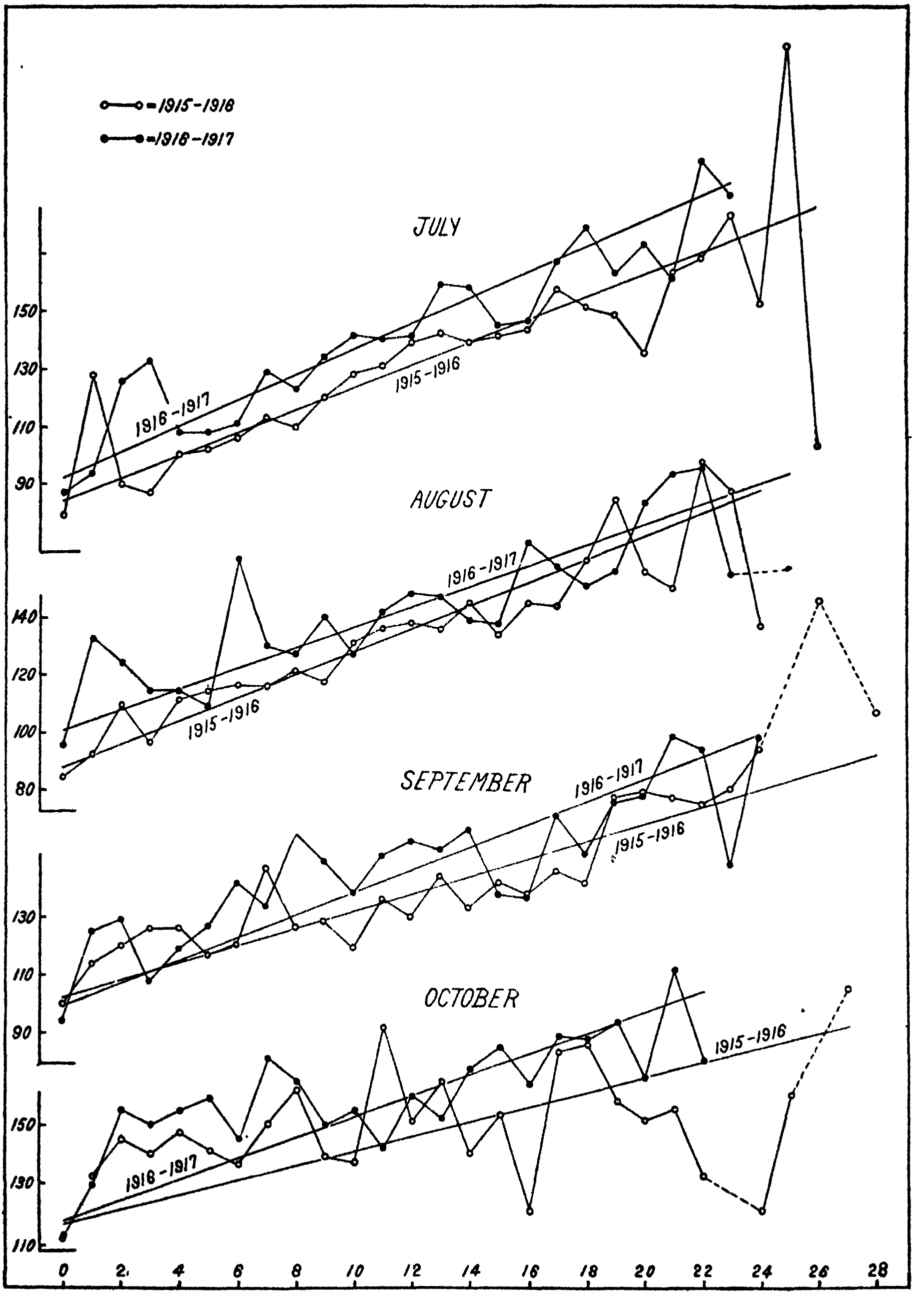

DIAGRAM 3.—Regression of annual production on monthly production.

The regression equations for the whole series of material, 1912 to 1919, are given in the final section of table 3.

These are not represented graphically in the present paper but will be given in a subsequent publication in which the results for Rhode Island Red birds studied in two different localities will be compared.

The correlation between the record of the individual month and that of the other eleven months of the year

Because of the fact that the production of each individual month of the year is included in the annual total production, the correlations between the productions of the individual months and the annual production must be expected to be positive in sign, and to some extent misleading as measures of the biological interrelationships between the different periods of the same year. This criticism will not apply to the correlations between the productions of the individual months and the total productions of the other eleven months of the year.

Calculating as we have in the White Leghorn series the correlation between the egg records of the individual months and the records of the remaining 11 months of the year, we obtain the results set forth in table 4.

These constants are lower throughout than those which have been deduced for the relationship between monthly production and annual production. This is shown clearly by diagram 4 in which the correlations for the production of each individual month and the production for the remaining 11 months of the year are compared with the correlations for

TABLE 4

Correlation between the monthly egg production and the record of the remaining eleven months of the year.

MONTHS	CORRELATIONS		
	1915-1916	1916-1917	1912-1913 to 1918-1919
November	.1822 ± 0310	.2990 ± .0292	.3218 ± .0149
December	.3110 ± .0289	.5256 ± .0232	.4456 ± .0133
January	.4046 ± .0268	.4341 ± .0260	4866 ± .0127
February	.3385 ± .0284	.4593 ± .0253	.4347 ± .0135
March	.3649 ± .0279	.4797 ± .0247	.4441 ± .0133
April	.4467 ± .0257	.3484 ± .0282	.3733 ± .0143
May	.4153 ± .0265	.3831 ± .0274	.3330 ± .0148
June	.4306 ± .0261	.4632 ± .0252	.4192 ± .0137
July	.4483 ± .0256	.4665 ± .0251	.4271 ± .0136
August	.4937 ± .0242	.4314 ± .0261	.4577 ± .0131
September	.3951 ± .0270	.5223 ± .0233	.4763 ± .0128
October	.2563 ± .0299	.3846 ± .0273	.3554 ± .0145

these months and the annual production. The result shows, however, that the correlation between the individual months of the year and the annual total is not due primarily to the inclusion of the individual month in the second variable of the correlation. The constants show clearly that birds which are superior in any individual month of the year, also, on the average, show superiority in the other 11 months of the year.

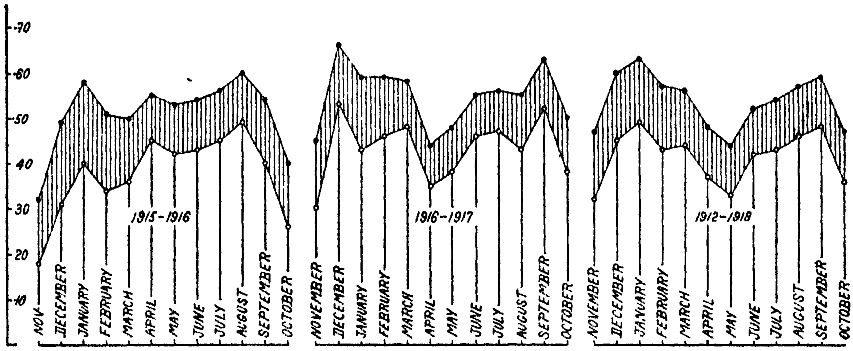

DIAGRAM 4.—Comparison of correlation between monthly production and annual production (solid dots) and between monthly production and the production of the remaining eleven months of the year (circles).

The correlation between the annual production and the deviation of the productions of the individual months from their probable values

In a study of the White Leghorn fowl (HARRIS, BLAKESLEE and KIRKPATRICK 1918, p. 46–49) the value of a coefficient showing the relationship between a variable and the deviation of a dependent variable from its probable value (HARRIS 1909) as a means of analyzing the interrelationship between the records of major and minor periods has been demonstrated.

The correlations between the annual record of the birds and the deviations of the contributions of the individual months to this annual record from their probable value appear in table 5 and are represented graphically in diagram 5.

These constants are also in excellent general agreement with those obtained from the first-year performance of the White Leghorn breed as exhibited at Storrs. The correlations show that November, December, January, February, August, September and October make relatively larger contributions to the annual production of superior birds than might be expected from the average results for the flocks as a whole. March, April, May and June make relatively far smaller contributions to the annual production of highly superior birds than might be expected from the average records. The relation of July production to annual

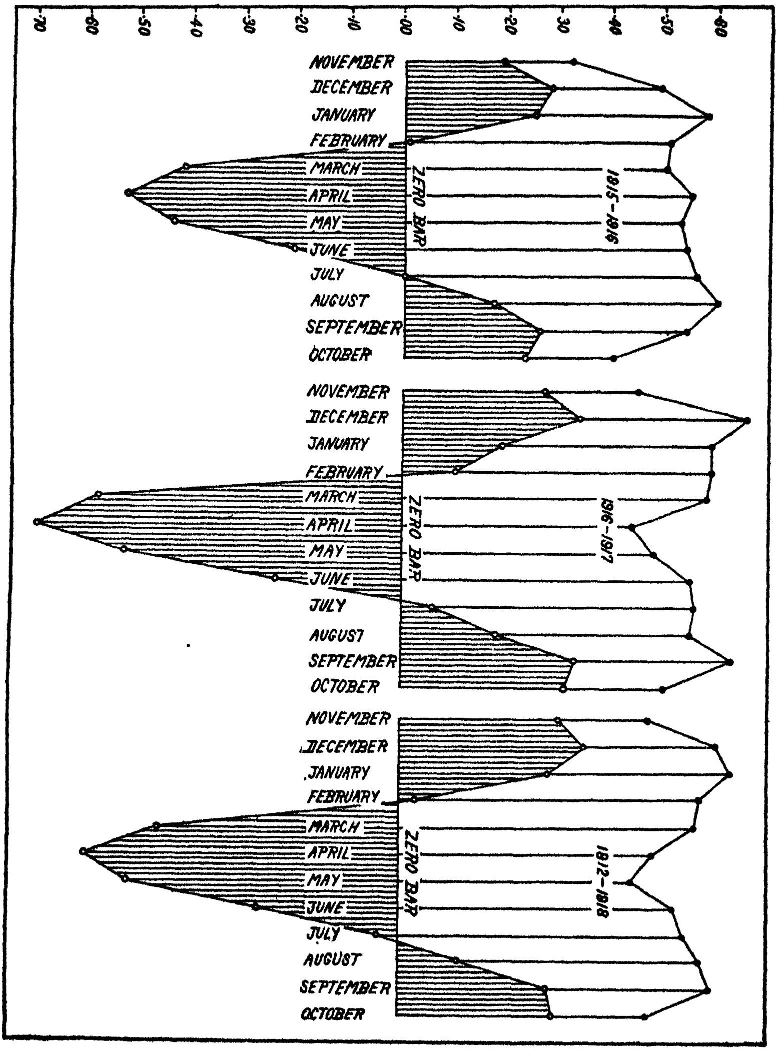

Diagram 5. Correlation between annual production and monthly production (solid dots), and correlation between annual production and the deviation of monthly production from its probable value.

production is somewhat questionable since it falls sometimes in one category and sometimes in the other.

While the coefficient for February is positive in all three series, it is numerically very small.

These results furnish another form of expression for the biological fact that during the months of March, April and May all birds are laying at about their maximum capacity. Large annual superiority cannot, therefore, depend primarily upon increased production in these months but must depend upon either precocious laying (November, December and January) or persistently continued laying (August, September and October), or upon both.

The correlation between the records of the individual months of the same year

Earlier studies (Harris and others, loc. cit.) have shown that in the White Leghorn there is a significant positive correlation between the records of each of five of the individual months of the first year and the records of each of the other eleven months of the first year. It has also been shown (Harris and Lewis 1922) that there is in general a positive correlation between the records of the twelve individual months of the first year and the twelve individual months of the second year in the White Leghorn fowl.

Table 5

Correlation between annual egg production and the deviation of the monthly records from their probable value.

MONTH	CORRELATIONS		
	1915-1916	1916-1917	1912-1913 to 1918-1919
November	+.1893 ± .0309	+.2667 ± .0298	+.3003 ± .0151
December	+.2781 ± .0296	+.3439 ± .0283	+.3471 ± .0146
January	+.2543 ± .0300	+.1915 ± .0309	+.2766 ± .0153
February	+.0066 ± .0320	+.1027 ± .0317	+.0275 ± .0166
March	−.4205 ± .0264	−.5814 ± .0212	− 4623 ± .0131
April	−.5261 ± .0232	−.6986 ± .0164	−.5951 ± .0107
May	−.4433 ± .0258	−.5307 ± .0230	−.5211 ± .0121
June	−.2138 ± .0306	−.2350 ± .0303	−.2748 ± .0153
July	+.0021 ± .0320	+.0639 ± .0319	−.0386 ± .0166
August	+.1660 ± .0312	+.1841 ± .0310	+.1147 ± .0164
September	+.2587 ± .0299	+.3264 ± .0287	+.2770 ± .0153
October	+.2287 ± .0304	+.3125 ± .0289	+.2922 ± .0152

We have now to consider the possible correlations between the records of the individual months of the first year in the three groups of Rhode Island Red birds under consideration.

The constants appear in tables 6 and 7. In table 6 the upper entry of the group of three represents the correlation coefficient for the 442 birds studied in 1916-1917 while the second entry gives the correlation coefficient for the 443 birds studied in 1915-1916. The third entry gives the difference and the probable error of the difference between these two coefficients obtained by taking (1916-1917) − (1915-1916). In table 7 the first of the three entries gives the correlation coefficients for the 1658 birds representing the whole period 1912 to 1919. The second and third entries represent the differences between the correlation as deduced from this larger series and those derived from the series for 1916-1917 and 1915-1916, respectively. These differences are so taken that the sign shows whether the single-year series gives a larger (positive sign) or a smaller (negative sign) correlation.

We now have to consider the consistency in the correlations deduced for the two years. This may be tested in three ways. First, we may determine the difference in the average value of the possible correlations between the individual months of the year in the two years for which constants are available.[2] Second, we may compare the homologous constants individually with regard to their probable errors. Finally, we may determine the magnitude of the correlations between the homologous correlations in the two years.

Consider first the difference in the mean values of the two series. We have

For 1916-1917,	.2448 ± .0694
For 1915-1916,	.1960 ± .0638
Difference	.0488 ± .0275

The average of the coefficients for 1916-1917 is slightly larger than that for 1915-1916, but the difference is only about twice as large as its probable error and cannot be asserted to be definitely significant.

For purposes of more critical comparison we must turn to the individual constants. The comparison of the individual coefficients for the two years has been made in table 6. Here the probable errors of the differences have been calculated in the usual manner.

It may be convenient to examine the frequency distribution of the differences. This is given in table 8. This shows that 19 of the 66 differences are negative, while 47 are positive in sign. This indicates clearly that the series for 1916-1917 is slightly more highly correlated than that for 1915-1916. In further evidence of this fact we may note that these are only four groups, each of 0.05 range, of negative differences, wherear there are seven such groups of positive differences.

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[2] We deal with the correlations deduced for two individual years because we are not certain of the method of computing the probable error of the difference if the constants for the two individual years were compared with those for the whole period 1912 to 1919. It is for this reason that probable errors are not given for the differences in table 7.

Considering the relation of the individual differences to their probable errors, as calculated by the usual formula, we have the frequency distribution shown in table 9. Here the frequencies of the various ratios to their probable errors are shown in groupings of unit range, the signs are those of the differences.

TABLE 8

DIFFERENCE IN CORRELATION (1916-1917) − (1915-1916)	FREQUENCY
−.151 to −.200	1
−.101 to −.150	4
−.051 to −.100	6
−.001 to −.050	8
+.001 to +.050	7
+.051 to +.100	21
+.101 to +.150	15
+.151 to +.200	1
+.201 to +.250	2
+.251 to +.300	
+.301 to +.350	1
Total	66

TABLE 9

RATIO TO PROBABLE ERROR AND SIGN OF DIFFERENCE	FREQUENCY
−4.001 to −5.000	1
−3.001 to −4.000	2
−2.001 to −3.000	4
−1.000 to −2.000	5
−0.000 to −1.000	7
+0.000 to +1.000	6
+1.001 to +2.000	19
+2.001 to +3.000	11
+3.001 to +4.000	7
+4.001 to +5.000	2
+5.001 to +6.000	1
+6.001 to +7.000	
+7.001 to +8.000	1
Total	66

We note that there are only five classes of negative ratios whereas there are eight classes of positive ratios. Again, it is clear that the frequency of large ratios is greater in the positive series. For example, there are only three cases in which the difference is over three times as large as its probable error in the negative differences, whereas there are eleven such cases in the positive differences. There are only seven cases in which the difference is more than twice as large as its probable error in the negative differences, whereas there are 22 such cases in the positive differences. It is clear, therefore, that the 1916-1917 series is somewhat more highly correlated than the 1915-1916 series.[3]

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[3] There was less disease in the flock during 1916-1917, and the range conditions were better for the growing birds than in 1915-1916. This may, or may not, be the reason for the differences in correlation.

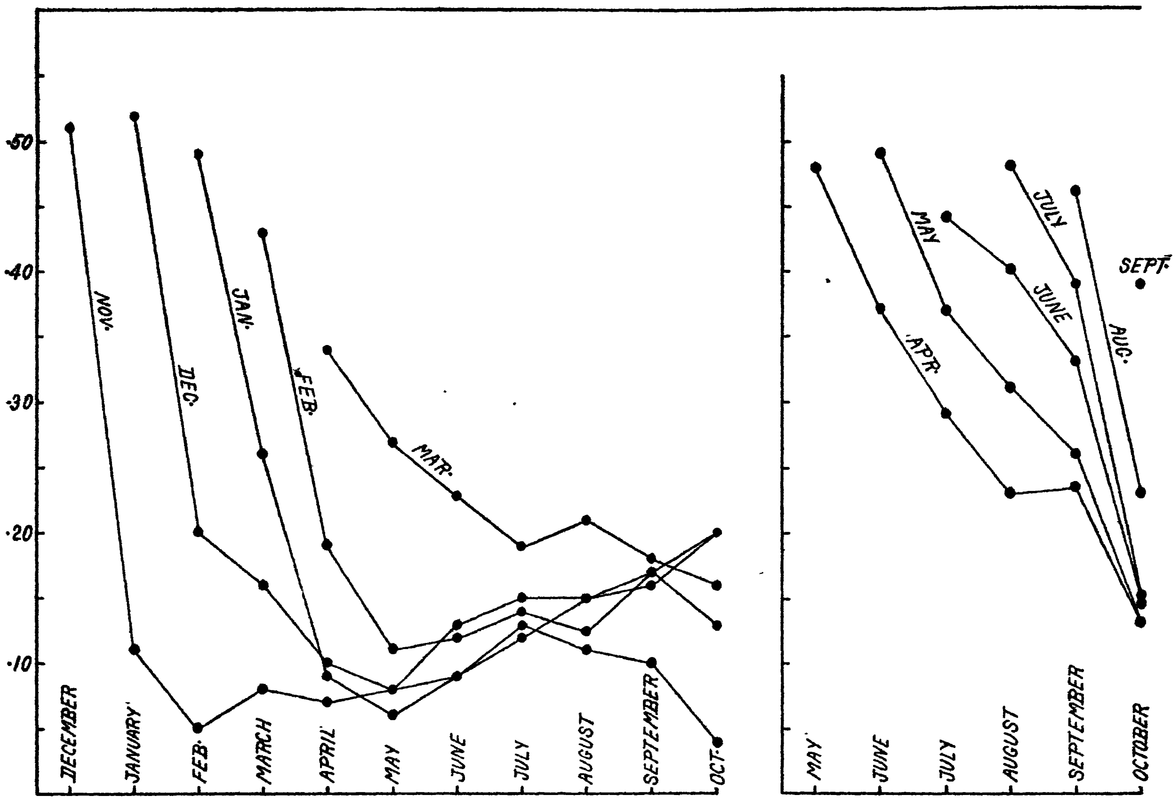

Diagram 6.—Distribution of magnitudes of correlations between the records of individual months and the records of subsequent months of the same year.

The foregoing analysis demonstrates that the correlation between the individual months of the 1916-1917 series is on the whole slightly higher than that for the 1915-1916 series. As a measure of the general consistency of the two systems of coefficients we may determine the correlation between the homologous coefficients for the two years. We deduce from the ungrouped data in table 6

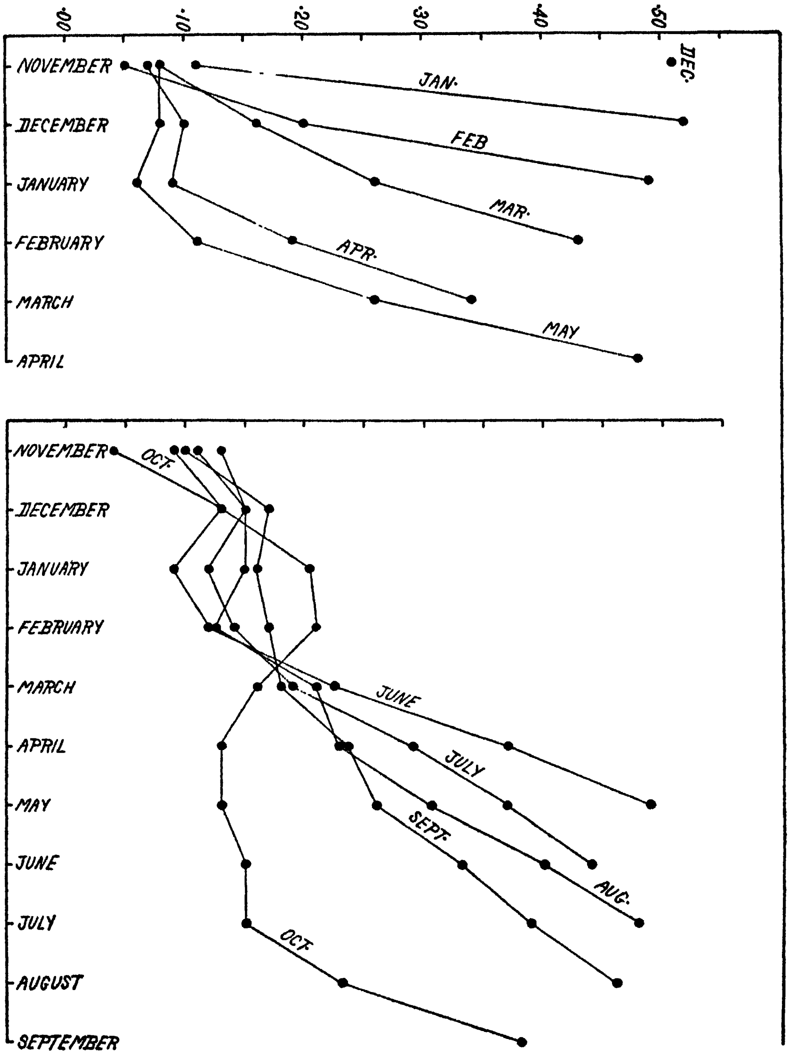

Diagram 7.—Distribution of magnitudes of correlations between the records of individual months and the records of antecedent months of the same year.

$$r = .9181 \pm .0130$$

This coefficient is very large indeed, and indicates a very close similarity between the coefficients measuring the relationship between the same combinations of months in the two years. It shows clearly that the correlations between the individual months do not differ in a merely haphazard manner but that there are definite and relatively rigid biological laws underlying these interrelationships.

When further data are available we shall have occasion to consider the relative magnitudes of the correlations between the correlations when birds of the same breed are compared at the same Station in different years, when the correlations for the same breed are compared at different Stations, and finally, when the correlations for different breeds are compared at the same Station or different Stations.

We now turn to a comparison of the magnitudes of the coefficients for the different combinations of months among themselves.

The reader will, of course, have noted that the mean value of the correlations is not large. It is only 0.244 for the series for 1916-1917, 0.196 for the series for 1915-1916, and 0.228 for the series for 1912 to 1919. The correlation between the series of correlations for the two years shows, however, that the coefficients for the different combinations of months differ among themselves. The significance of these differences in the magnitudes of the coefficients may best be brought out by graphic presentation.

The constants are divided into two series, the first (diagram 6) showing the relationship between the record of each month and that of each subsequent month, the second showing the correlation between the record of each month and that of each antecedent month (diagram 7).

On these diagrams, the verticals represent the subsequent or the antecedent months of the year as the case may be. The magnitude of the correlation between the production of any month (as indicated by the lettering on the lines connecting the dots for this month) and that of the months of the year is indicated on the scale of ordinates on the vertical for the antecedent or subsequent month in question.

In the preparation of these diagrams, it has seemed desirable to smooth out in so far as possible the probable errors of random sampling by using the averages of two independently determined constants. The values represented are the averages of the two series of correlations deduced from the 1915-1916 and the 1916-1917 series. The distribution of the several months between the two sections of the diagrams has been determined by clearness of presentation merely.

These diagrams show that in the Rhode Island Red as in the White Leghorn breed of poultry we may recognize the existence of two laws underlying the interrelationship between the productions of the individual months of the first year. These laws have been stated (HARRIS, BLAKESLEE and KIRKPATRICK 1918, p. 70–71) as follows:

> "First, the correlation between the egg production of the individual months tends to become smaller as the months considered are more widely separated in time.
>
> "Second, there is a more intimate correlation between the egg production of the autumn and winter months at the beginning and end of the contest year than between the egg production of these months and the productions of the spring and summer months."

Referring to the diagrams for details, we note from diagram 6 that for November, December, January and February production there is at first a rapid decline in the magnitude of the correlations with successively later subsequent months until a minimum value is reached for the production of the month under consideration and that of April or May. There is then an increase in the correlations for the production of these months and those of the months of June, July, August, September and October. The correlations for March are an exception to the other four months represented on this figure, in that correlation decreases as the successive months are more widely separated. This is also true for all of the months, April to August, represented on the second section of diagram 6.

Turning to the relationship between the egg production of individual months and that of preceding months, we note from diagram 7 that the correlations between January, February and March production and the production of preceding months decreases as the months become more widely separated in time. The correlations for April, May, June, July, August and September show a slight increase for the relationship between the production of these months and that of the earlier months of the year. The curve of the correlations of October production with that of the earlier months illustrates in a striking manner the existence of the two laws.

The comparison of the results for the Rhode Island Red breed with other breeds of poultry is reserved until the other constants, computed but not yet published, are available.

RECAPITULATION

This paper presents the results of a statistical analysis of the monthly egg records for the pullet year of 1658 Rhode Island Reds as recorded at the MASSACHUSETTS AGRICULTURAL EXPERIMENT STATION, Amherst, Massachusetts.

The following problems are considered:

1. The correlation between monthly record and annual production. The coefficients are shown to have a material positive value ranging from 0.325 to 0.634 in the 36 determinations made. The average values are $\bar{r}=0.5094$ for 1915-1916, $\bar{r}=0.5479$ for 1916-1917, and $\bar{r}=0.5363$ for 1912 to 1919.

These coefficients are of about the same order of magnitude as those already demonstrated for White Leghorn fowl (Harris and others, 1917, 1918). The regression of annual egg production on monthly egg production is, roughly speaking, linear. Prediction of annual egg production of groups of birds may therefore be made with relative ease from the records of an individual month by the use of equations of the type computed and shown to be effective for the White Leghorn breed (Harris, Kirkpatrick and Blakeslee 1921; Harris, Kirkpatrick, Blakeslee, Warner and Card 1921).

2. The correlation between monthly record and the total record of the other eleven months of the year. These values are, as might be expected, lower than those for the relationship between monthly production and annual production. They are, however, positive in sign and statistically significant throughout. They show, therefore, that the correlations between the records of the individual months and annual total is not due primarily to the inclusion of the record of the individual month under consideration in the annual total.

3. The correlation between the annual record and the deviation of the production of the individual months from their probable value. These coefficients show that the several months of the year differ greatly in their relative contribution to the annual egg record of unusual birds.

4. The correlation between the record of the individual months of the same year. All possible values of the correlation between the records of the individual months of the first year have been determined for two years individually and for the records for the entire period 1912 to 1919.

These coefficients are on the average small. The average value of the correlation is $\bar{r}=0.196$ for 1915-1916, $\bar{r}=0.244$ for 1916-1917, and $\bar{r}=0.228$ for the entire period, 1912 to 1919. The coefficients for the different permutations of months differ widely among themselves. This is shown by the fact that there is a correlation of .918 between the system of coefficients for 1915-1916, and that for 1916-1917. In their distribution the coefficients show the existence of two laws. First, the coefficient between the egg production of the individual months tends to become smaller as the months considered are more widely separated in time.

Second, there is a more intimate correlation between the egg production of the autumn and winter months at the beginning and end of the pullet year than between the egg production of these months and the productions of the spring and summer months. These two laws of the distribution of the magnitude of the correlations are to some extent mutually obscurant.

The foregoing conclusions are in general agreement with those already drawn for White Leghorns. Detailed comparisons between the more important commercial breeds will be made in a subsequent paper.

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STUDIES ON INHERITANCE IN PIGEONS. IV. CHECKS AND BARS AND OTHER MODIFICATIONS OF BLACK[1]

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INTRODUCTION

A considerable interest has for some time been attached to the evolutionary, and in later years to the hereditary, relationships of the two color patterns in pigeons, known as blue black-barred (figure 5) and check (figure 3). This was due, in part at least, to the probable universality of these two patterns in many of the old domesticated races. According to BUFFON (1793), for instance, ALDROVANDUS about 1599 described the pigeon of Crete as having a bluish plumage, "and marked with two blackish spots on each wing." The same authority quotes WILLUGHBY (1678) as giving a similar description for the pigeon of Barbary. MOORE (1735) occasionally mentions "blue" types among the several varieties listed by him, as for example the Spot, the Jacobin and the Helmet. Both blue and checked examples are referred to in the "Taubenbuch" (ANONYMOUS 1790). Though the descriptions of these early writers are too brief to show with certainty that the "blue" to which they allude is similar to the blue with black wing bars, or blue with black check-marks of modern breeds, there is nothing to indicate a reference to any other color.

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Writers of the early and middle nineteenth centuries, as BOITARD and CORBIE (1824) and EATON (1858), also mention blue and checked varieties of several breeds, describing them in detail. Later DARWIN (1868, vol. 1, p. 197) listed several breeds in which these two color patterns were known to occur.

Of equal if not even more importance than the mere presence of barred and checked types in established breeds was the occasional appearance of these two patterns, not only in presumably "pure" stocks of other colors, as black or white, but also in crosses of various colored birds not having, so far as was known, any barred or checked individuals in their ancestry. Descriptions of some such crosses made by the writers just cited, are presented by them in some detail. It will be sufficient to state here, however, that special attention was called to the unexpected cropping out of birds with one or the other of the patterns under discussion. As late as 1909 the French writer BAILLY-MAITRE refers to blue as the fundamental color and calls attention to its unexpected occurrence in various stocks.

At the same time the presence of the barred pattern among different species of pigeons in widely separated geographic areas was noted and described. DARWIN (1868, vol. 1, pp. 182–184) and TEGETMEIER (1868, pp. 26–27) describe these various species. The first one is *Columba leuconata*, which is blue with three wing bars and inhabits the Himalayas; those with two wing bars are *C. livia*, found on the coasts of Scotland and England, and *C. rupestris* of central Asia. Similar to these are *C. schimperi*[2] of Abyssinia and *C. gymnocyclus* of West Africa. These and others are detailed by SALVADORI (1893). More might be enumerated but the above will suffice to show the prevalence of the blue black-barred pattern among various species of the Columbidae.

Checked varieties for each of these wild species are not mentioned, but they may nevertheless exist. Such a variety of *C. livia* is well known and Darwin (1868, vol. 1, p. 184) mentions a checked specimen of *C. schimperi* in the BRITISH MUSEUM.

Somewhat similar checked patterns are found in *C. guinea* and many of the doves (mourning doves, ground doves, etc.); in fact a large number of the individuals of the order Columbae exhibit checks. Whether the genetic behavior of the checks of *C. livia* and of the doves are similar is as yet a question.

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[2] The writer examined a blue specimen of *C. schimperi* belonging to the FIELD COLUMBIAN MUSEUM of Chicago and was struck with its similarity to our common blue pigeons. This specimen was from Egypt.

From a study of the above-named species and many more DARWIN was led to the belief that *C. livia* was the progenitor of the domesticated pigeon, since it resembles the domesticated varieties in form more closely than do the other species. But of equal importance is the fact that it is easily domesticated and proves to be fertile with and to produce fertile hybrids by the domesticated pigeon.

Granting *C. livia* to be the progenitor of the domesticated races DARWIN easily accounted for the reappearance of checked and barred birds in supposedly pure stocks and in certain crosses, as reversions to the ancestral type. It is still generally conceded that *C. livia* is the progenitor of the domesticated varieties, and geneticists also interpret the unexpected appearance of checked and barred birds as reversions, but they attribute them to factor recombinations (COLE 1914, p. 343).

After conceding *C. livia* to be the ancestor of the modern races of pigeons, the next step was to determine the relationships of the two patterns, check and bar, and to discover if possible which was the original. It is this question which has produced a certain amount of speculation and some experimentation from the evolutionary and genetical standpoints.

DARWIN believed the barred type to be the original, while checked birds were probably due to "the extension of these black marks [black bars] to other parts of the plumage" (DARWIN 1868, vol. 1, p. 183). WHITMAN, on the other hand, assumed the opposite view, namely that the barred type resulted from a gradual clearing of the wings of checks "from before backwards" (WHITMAN 1919, vol. 1, p. 19), until the two-barred type resulted. He went further and stated that the evolution of these types was an orthogenetic one, basing his conclusions on certain results which he obtained from selection experiments.

The above are the two main evolutionary views, which obviously contradict each other. The other way of ascertaining the relations of the two patterns is by Mendelian breeding methods applied both to domesticated varieties and to birds of the wild stock representing the two patterns. These genetic relationships have been fairly well established by STAPLES-BROWNE (1908, 1912) and BONHOTE and SMALLEY (1911), who advanced data showing the independent Mendelian inheritance of the two patterns.

In view of the foregoing, the object of this paper is to show the hereditary relationships of the two types under consideration to the other colors in pigeons, with special reference to black; secondly, to show from a microscopic study of pigment arrangements and breeding experiments the interaction of several factors affecting blue and black; and lastly to interpret WHITMAN'S results on a genetic basis.

The data which follow are taken not only from special matings made by the writer at the WISCONSIN AGRICULTURAL EXPERIMENT STATION during a period of three years, but also from the entire set of pigeon records dating back to the beginning of the work by Professor COLE at the RHODE ISLAND STATION in 1907. As a consequence many of the matings used by him in his 1914 paper and also in the 1919 paper by COLE and KELLEY are repeated here.

THE "BLUING" SERIES

There is a wide range in the amount of blue which may occur, from full black, where no blue at all is present, to a type known as barless, in which nearly the whole bird is blue. The variations form a series of gradations of increasing "bluing." The six types which have been distinguished in this work are fairly distinct, though there is some intergrading in certain cases. The breeding behavior, however, seems to indicate their genetic distinctness. It is probable that the intergrading is due, in some cases at least, to modifying factors of one sort and another; and it is also not improbable that other genetically distinct grades may exist besides those described. These six types are described in the order of their progressive bluing (decreasing amount of black in relation to blue), and it is interesting that they appear to be epistatic in their hereditary manifestation in the same order, i.e., each to the one that follows. It is to be noted that blue appears in the rump and in the outer vanes of the outer tail feathers independently of this series.

"Full black" (figure 1) as used in the present paper refers merely to the absence of blue everywhere except in the rump and outer vanes of the outer tail feathers. Blue appears first in the tail, making the "black blue-tail," and its further invasion results in the "checked," "sooty," "blue black-barred" and "barless" types, all of which, however, have the characteristic blue tail. Full black takes no cognizance of kitiness (reddish), often present in the flights of black pigeons, nor of white spotting, nor, as mentioned above, of a dark blue rump or light outer vanes of outer tail feathers. A bird might possess all these characteristics, but so long as it lacked the blue tail with black terminal band it would be classified as a full black.

"Black blue-tail" is the next stage below full black. The chief distinguishing characteristic from black is the blue tail with a black terminal band. This pattern shows variability in itself, since all birds entirely black except for blue tails (figure 2) to those with blue or almost white rumps and with or without some bluing in the primaries, but with unchecked wing coverts are included in this class.

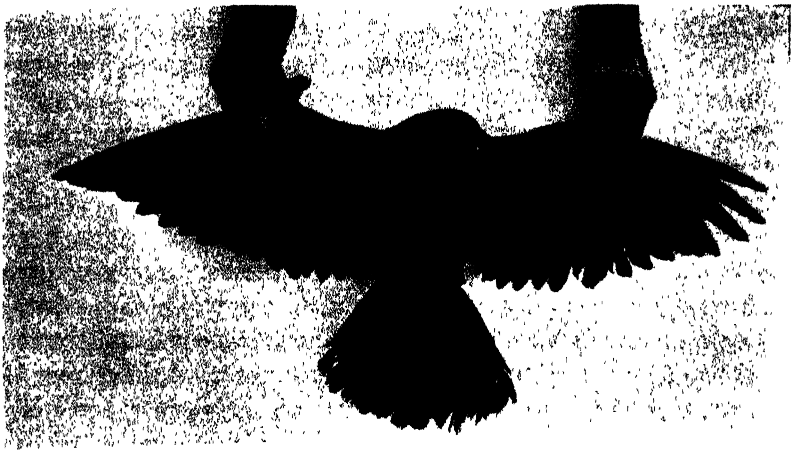

Figure 1.—Full black with uniform rump (grade 5) and uniform outer vanes of outer tail feathers. (Wisconsin No. 1673A.)

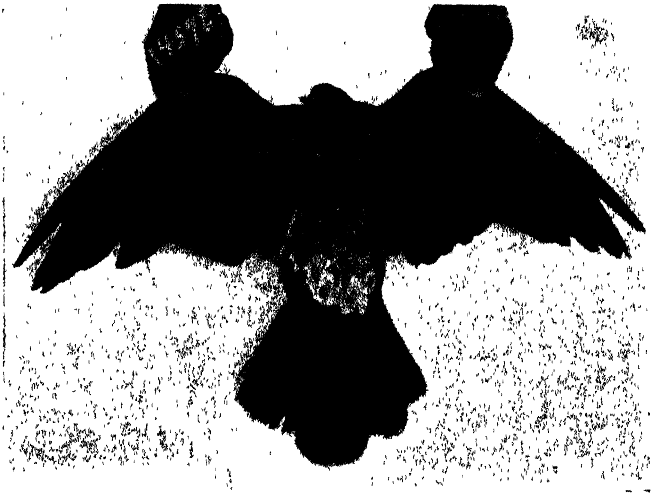

Figure 2.—Black blue-tail with uniform blue rump (grade 3) and light outer vanes of outer tail feathers. The white feathers in the rump are not considered. (Wisconsin No. 1457E.)

References to this type are not very numerous. DARWIN (1868, vol. 1, p. 184) in describing the Rock Pigeon of Madeira says, ". . . others are chequered, like *C. affinis* from the cliffs of England, but generally to a greater degree, being almost black over the whole back; . . . . ." The "reversionary blues" described by STAPLES-BROWNE (1908, 1912) belong in this class.

"Check" originates from the so-called checked appearance of the wing (figure 3). This condition is caused by the presence of two black marks situated respectively in the inner and outer vanes of the wing coverts, the central and proximal portions of which are blue. The rest of the plumage of checked birds is blue with the invariable exception of a black terminal band on the tail and of the not infrequent presence of checks on the upper back. The rump may be either very light, almost white, or a shade of blue uniform with that of the wing coverts, or any shade between these two colors.

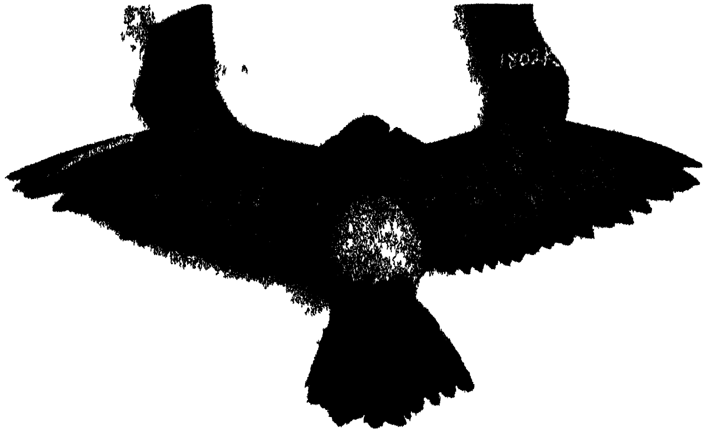

FIGURE 3.—Medium check with extreme light blue rump (grade 1); outer vanes of outer tail feathers only slightly lighter than those of the other tail feathers. (Wisconsin No. 1802K.)

"Sooty" is the fourth variation of the bluing series to be considered. The black blue-tail represents a character which is blacker than the check, while the sooty character is representative of the opposite condition. Sooty, as the name implies, comprises those individuals whose wing coverts have a mere sprinkling of black, giving the bird a sootied appearance (figure 4). The black on the wing coverts is so small in amount that it fails to take on a definite pattern as it does in the typical check.

It is not at all difficult to differentiate between sooty and check, as the sprinkling of black on the wing coverts is easily distinguished from the definite black spot of the check mark.

References to this type are also few. CHAPMAN (1911, p. 5) mentions sooty blues. STAPLES-BROWNE (1912) in describing the offspring from the Rock-Fantail cross says that the classification of birds into "chequered" and "non-chequered" groups was complicated by several individuals which he termed "intermediates" and described "as showing a slight darkening of some of the lower wing coverts, which is not noticeable unless the bird is caught." This description is in keeping with what is referred to as sooty in the present paper. Although WHITMAN does not definitely discuss this character, the plate of *C. oenas* (WHITMAN 1919, vol. 1, plate 9) appears to depict the character very well.

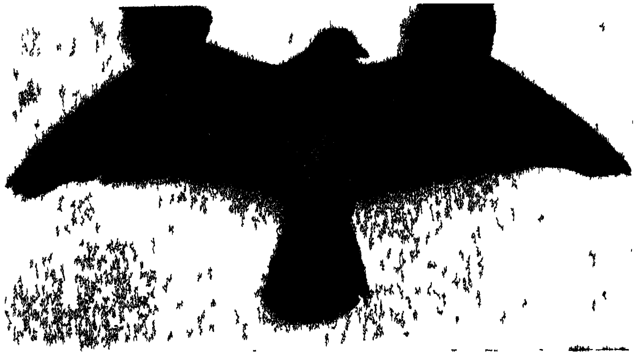

FIGURE 4.—Sooty blue with light blue rump (grade 2) and light outer vanes of outer tail feathers. (Wisconsin No. 1777A.)

"Bar" refers to the pattern commonly called "blue black-barred." The wings of a barred bird are blue with two transverse black wing bars, one of which extends through the tertiaries and innermost secondaries, while the second extends through most of the secondary coverts (figure 5). The so-called blue of the pigeon is in reality a gray which, according to COLE (1914), corresponds to RIDGWAY's "gull gray." The tail and rump are similar to those of the check, but the back is free from any check marks.

"Barless," the sixth and last variation, is, so far as known at present, the lowest stage in the bluing series. As the name implies, the wings lack any bars, which makes the distinction between this type and bar very easy. In the barless individual the so-called blue has entirely replaced the black wing bars leaving a clear blue wing, except for the darkened tips of the primaries, secondaries and tertiaries.

This character has been known for some time. DARWIN (1868) mentions as barless a sub-variety of the Swallow, of German origin, pictured by NEUMEISTER. LYELL (1887) calls attention to the same pattern in the Ice Pigeon and a variety of the Swiss Pigeon. SCHACHTZABEL (1906) pictures several barless varieties as follows, the Strasser, Goldgimpel, (which has a yellow body and blue barless wings), the Pfaffen and Eichbuhlertaube. BAILLY-MAITRE (1909) also names a barless variety of German origin, "Feld-Tauben." Both the barless Strasser and Ice Pigeon as well as barless varieties of the Swallow and Homer are produced in this country. Figure 6 represents the latter breed.

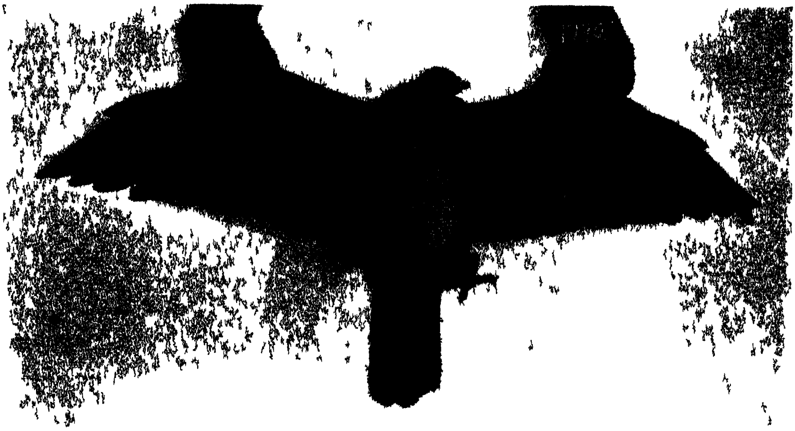

FIGURE 5.—Blue black-barred with light blue rump (grade 2). (Wisconsin No. 1790A.)

Genetic relationships of black, check and bar

The phenotypic differences between these three characters depend upon the presence of varying amounts of the two so-called colors, black and blue, the check being the intermediate type. A further difference between the two colors is one of pigment-granule arrangement. COLE (1914) showed these granules in the barbules of a black feather to be evenly distributed and those in a blue feather to be clumped. These two conditions have been found, as might be expected if checks are a combination of black and blue, existing side by side in the feathers of checked birds. It is this difference in pigment arrangement that produces the two different optical effects.

Cole also showed that full black is dominant to the barred type. In view of the microscopic study just discussed he postulated a factor *S* which referred to the spreading of the pigment granules evenly throughout the barbules of black feathers, while *s* indicated the clumped condition in blue ones. Table 1 is presented as furnishing further data on the dominance of black birds known to be *SS* to bar (*ss*). From three such matings 22 full-black offspring resulted. Cole's data and those of the writer regarding the segregation of the *S* factor will be presented and discussed later. It may be stated here, however, that black differs from bar by a single factor.

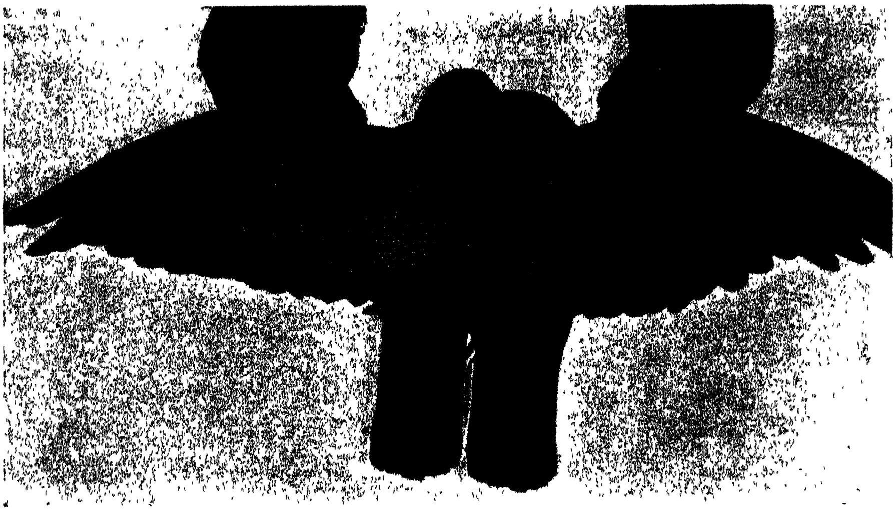

Figure 6.—Blue barless Homer with light blue rump (grade 2). (Wisconsin No. 1751A.)

Table 1

Black (SS) by bar (ss).

MATING	♂♂	COMPOSITION	♀♀	COMPOSITION	OBSERVED *S*-BLACK	EXPECTED *S*-BLACK
1033	999A	*sscc*	7A	*SS*(*CC*)*	10	10
1746	1671A	*SS*(*CC*)	1470F	*sscc*	7	7
1803	1462H	*SS*(*CC*)	1670A	*sscc*	5	5
Total (3 matings)					22	22

* Letters inclosed in parentheses indicate uncertainty as to this portion of the formula.

The next step was to establish the relation of black to check. Since the black birds in the colony at the Wisconsin Station were known to be heterozygous for various color characters including those for check and bar, the purchase was made of several black Tumblers, presumably "pure" for black, that is, not having any barred or checked ancestry. The checked parents were from colony stock and were known to be heterozygous. The breeding results proved the blacks purchased to be homozygous, and it is obvious that black is dominant to check, since in six matings 57 full-black birds were obtained, and as many as 22 in a single mating (1686).[3]

When two blacks homozygous for *S*, or when at least one parent is of this composition, are mated together, only full blacks result. Six such matings gave a total of 61 full-black offspring.

That there is apparently a single-factor difference between black and check is shown by the very close 3 : 1 ratio obtained in table 2. Five matings of full-blacks to full-blacks when both were known to be heterozygous give a total of 25 blacks to 8 checks, the expected being 24.75 to 8.25.

TABLE 2

Segregation of S and s in presence of CC.*

MATING	♂♂	COMPOSITION	♀♀	COMPOSITION	OBSERVED		EXPECTED	
					S Black	*C* Check	*S* Black	*C* Check
554+	450X	*SsC(C)*	450Y	*SsC(C)*	6	1	5 25	1.75
662+	493B	*SsC(C)*	454B	*SsC(C)*	8	1	6.75	2.25
1015+	790B	*SsC(C)*	799A	*SsC(C)*	4	1	3.75	1.25
1025+	862A	*SsC(C)*	862B$_3$	*SsC(C)*	3	3	4.50	1.50
1812	1685C	*SsC(C)*	1594Q$_8$	*SsC(c)*	4	2	4.50	1.50
Total (5 matings)					25	8	24.75	8.25

* Only those matings from which 5 or more offspring resulted, and only those for which complete descriptions were made, have been used in the compilation of this table.

Having shown black dominant to both check and bar and differing from each by a single factor, the relation of check to bar will next be discussed. BONHOTE and SMALLEY (1911) state that they have accurate records on fifty-seven matings of check by check, from which 229 checked offspring resulted. Reference to the work of STAPLES-BROWNE (1912) gives one mating of check by check producing ten offspring which, according to the author, indicates that at least one parent was homozygous for check. He also presents a mating of check by bar giving eight checked squabs, indicating the dominance of check to bar. Similar data are available from WHITMAN (1919, vol. 2, table 72, p. 104). Two checked wild Rocks produced 16 checked offspring and no barred, showing without doubt that one parent at least was homozygous for check. In the present experiments a mating (1607) of homozygous check by bar gave 8 checked offspring and two others (1596, 1743) of a black blue-tail male, known to be homozygous for *C*, with barred females produced 12 check and 2 black blue-tailed progeny. This male must have been heterozygous for black blue-tail. These and the earlier results demonstrate that checks (or black blue-tails) homozygous for *C*, when mated to checks or bars, do not throw individuals lower in the series than checks.

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[3] Many of the tables giving detailed results of individual matings have been omitted from this paper in order to economize in the cost of printing. They will be found in full in the original thesis deposited in the Library of the UNIVERSITY OF WISCONSIN.

Data on the segregation of the factor for check are also numerous. STAPLES-BROWNE gives three matings of check by check in which both parents are heterozygous. The result of these matings was 21 check to 12 bar, which is not very close to a 3:1 ratio. BONHOTE and SMALLEY (1911) give two similar matings from which 13 checked and 4 barred offspring were obtained, which numbers are very close to the above ratio. The work of NUTTAL (1918) might offer some available material on this point if the matings had not been complicated with the known sex-linked *A*-factor which restricts black, and possibly with others. WHITMAN (1919, vol. 2, table 72, p. 104) obtained from two matings of checked birds, presumably heterozygous judging from the patterns of the progeny, 23 checked offspring and 5 barred. One of the parents of one mating was procured from a fancier and the other parent was a stray pigeon, while the parents of the second mating were both original wild Rocks from the Cromarty caves of Scotland. These figures also, considering the numbers, fit a 3:1 ratio fairly closely. Eleven matings made by the writer, in which checked birds heterozygous for this factor were interbred, gave 59 checked (excluding 1 possible black blue-tail) to 18 barred offspring. This is very close to a 3:1 ratio (57.75 : 19.25). A summation of the foregoing results gives 116 checked to 39 barred birds, where the expectation is 116.25 to 38.75, seeming to demonstrate that check and bar differ by a single Mendelian factor.

Further data, from matings in which 1:1 ratios are expected, are available from STAPLES-BROWNE and WHITMAN. The latter presents two such matings giving 9 checks to 10 bars, and the former (STAPLES-BROWNE 1912) two matings which give 5 checked to 18 barred offspring. The results of the last two matings are certainly not very close to expectation. A tabulation of twenty-one matings of heterozygous checks mated to bars, exhibits a fair 1:1 ratio (87:105).[4] $\frac{\text{Deviation}}{\text{Probable error}}\left(\frac{.09}{.05}\right)=1.80$ indicates that the discrepancy may be due to random sampling.

Blacks, then, throw blacks, checks, and, as will be shown presently, also bars, while checks throw only checks and bars. Bars, however, produce neither blacks nor checks but bars only, as is shown by the following results obtained from many matings in several stocks of bars to bars. Bonhote and Smalley (1911) secured from twelve such matings 41 barred offspring, Staples-Browne (1912), 198 barred birds, and Whitman (1919), 16 barred individuals from six matings of barred wild Rocks. The writer's data show that forty-nine barred offspring resulted from six matings of bar to bar and one mating of bar to barless (1770). Three hundred and four barred birds have, accordingly, been obtained from at least four entirely different stocks constituting 53 different matings of bars to bars, which shows fairly conclusively that barred birds breed true.

In order to explain the foregoing results a factor C for check has been postulated. It is not linked with S but can act only in the presence of s to produce the checked pattern. In other words S is epistatic to C. When neither S nor C are present the barred type is the result. Blacks, then, may or may not carry C and can consequently be of the following compositions, $SSCC$, $SSCc$, $SScc$, $SsCC$, $SsCc$, $Sscc$. Checks, on the other hand, can be of but two sorts, $ssCC$, $ssCc$, while the barred type can be only $sscc$.

In order to further illustrate such a hypothesis the data in tables 3 and 4 are presented. Table 3 shows four matings of blacks by blacks heterozygous for S and C, in which case a 12:3:1 ratio is expected. The total for each of the three classes for which complete descriptions are given is small (15:6:4). As a consequence the closeness of fit (P) is poor (.086). When, however, all individuals are included the ratio becomes 25:6:4 and the value for P is then .461 which is a fair fit. The matings of table 4 consist of black birds heterozygous for S and C, mated to bars, which give an expected ratio of 2:1:1. The check class is low and the bar class high. Consequently the value for P (.154) can hardly be called fair. It is reasonable to suppose that the low values for P are due to the small numbers from which they are calculated and not to a misinterpretation of the data.

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[4] The 105 bars include 4 sooty birds, which can be legitimately included in the present instance, however, as "non-checks."

TABLE 3

Blacks (SsCc) by blacks (SsCc).

MATING	♂♂	COMPOSITION	♀♀	COMPOSITION	OBSERVED			EXPECTED			NOTES
					S Black	*C* Check	*Ba* Bar	*S* Black	*C* Check	*Ba* Bar	
1296	1033A	*SsCc*	1033B	*SsCc*	4	2	2	6.0	1.5	.5	2 blacks not included, poor descriptions
1305	1033D	*SsCc*	1033E	*SsCc*	7	2	1	7.56	1.89	.63	3 blacks not included, poor descriptions
1110+ and 1332	905B	*SsCc*	864A	*SsCc*	2	1	1	3.00	.75	.25	4 blacks not included, poor descriptions
1126+ and 1311	962A	*SsCc*	915B	*SsCc*	2	1		2.28	.57	.19	1 black not included, poor description
Total (4 matings)					15	6	4	18.72	4.68	1.56	$\chi^2=4.924$ P = .086
Total all birds included					25	6	4	26.28	6.57	2.19	$\chi^2=1.609$ P = .461

TABLE 4

Black (SsCc) by bar (sscc).

MATING	♂♂	COMPOSITION	♀♀	COMPOSITION	OBSERVED			EXPECTED			NOTES
					S Black	*C* Check	*Ba* Bar	*S* Black	*C* Check	*Ba* Bar	
1448	1341D	*sscc*	915B	*SsCc*	8		4	6.00	3.00	3.00	2 not used
1454	1385B	*SsCc*	1242A	*sscc*	6		4	5.00	2.50	2.50	1 not used
1484	962A	*SsCc*	1432A	*sscc*	1	2	2	2.50	1.25	1.25	
1681	1512R	*SsCc*	1657Z	*sscc*	1	1	2	2.00	1.00	1.00	1 not used
1682	1448T	*SsCc*	1668A	*sscc*	4	5	4	6.50	3.25	3.25	
1742	1666G	*sscc*	1454V	*SsCc*		1	2	1.50	0.75	0.75	
1748	1657J	*sscc*	$1616A_2$	*SsCc*	5	1	1	3.50	1.75	1.75	
1767	1532D	*sscc*	1699Q	*SsCc*	7	3	5	7.50	3.75	3.75	
1824	1659S	*sscc*	1605V	*SsCc*	2	1	1	2.00	1.00	1.00	
1859	$1553J_2$	*SsCc*	1657Z	*sscc*		1	1	1.00	0.50	0.50	
Total (10 matings)					34	15	26	37.50	18.75	18.75	

$\chi^2=3.782$ P = .154.

The description of the inheritance of these three patterns corresponds somewhat to that offered by COLE (1914) for the occurrence of STAPLES-BROWNE's "reversionary blues." COLE suggested a factor *T* which, like the *C* factor, acted only in the absence of *S*. He also pointed out that the blues which STAPLES-BROWNE recovered from his second generation of black Barb and white Fantail cross were not the same as those to which DARWIN referred. These birds instead of having clear blue wings with two black wing bars as described by DARWIN and referred to in this paper as the barred type, had black wings with indistinct blacker bars and blue tails with black terminal bands. In offering such an explanation for STAPLES-BROWNE'S data, COLE differentiated between typical full blacks and blacks with blue tails, as the writer has done for blacks and checks, but in presenting his own data on the segregation of the *S* factor all black blue-tailed birds and also checks were included in one class with the full-blacks, while the typical blue black-barred birds were put in the other class. Data have been presented to show that blacks and checks differ by the *S* factor and more data will be given later to show that black blue-tailed birds do not carry *S*. Consequently the *S* factor as presented here differs from that postulated by COLE for his own data, but corresponds to that offered by him in explanation of STAPLES-BROWNE'S findings. According to the present interpretation it refers only to full-blacks, while all black blue-tailed and checked birds are the result of its allelomorph (*s*) plus two other factors, *T* and *C*. On such a hypothesis COLE'S data showing the segregation of *S* should better fit a 15:1 ratio than a 3:1. While his numbers (19:3) based on the offspring from three matings are too small to prove this point one way or the other, they at least fit a 15:1 ratio quite as well as a 3:1.

The epistatic series, black, black blue-tail, check, sooty, bar and barless

The relation of black to check and bar has been discussed. It is now proposed to consider the other members of the series.

Data giving the complete Mendelian inheritance of black blue-tail are insufficient. What there are, merely show that it appears to behave toward black very much as does check. Seven matings of blacks to blacks which throw black blue-tail, gave 21 of the former to 9 of the latter. It may be assumed that these blacks were heterozygous for *S* and homozygous for *T*, the factor determining black blue-tail, since they produced no offspring lower in the series. This result is suggestive of a single-factor difference.

Various matings of black blue-tail to other types in the series are given in sections A, B, C and D of table 5. In the later sections are various combinations of other types lower in the series. The numbers in most cases are too small to have much significance as to ratios, but it is to be noticed that in general the results confirm the conclusion that in any mating offspring are not produced higher in the series than the high parent. The one or two exceptions will be discussed below. In many of the matings there were offspring which died too young for description, or which were for other reasons insufficiently described; these might have changed the ratios materially.

Section A consists of two matings of blacks by black blue-tails. Three types are produced, blacks, black blue-tails and checks. In section B there is an exception to the rule that the grade of the offspring is not higher than that of the parents, for a pair of black blue-tail birds have produced three full-black offspring. By hypothesis black blue-tails do

TABLE 5

Matings of blacks, black blue-tails, checks, sooties and bars.

MATING	♂♂	COMPOSITION	♀♀	COMPOSITION	*S* BLACK	*T* BLACK BLUE-TAIL	*C* CHECK	S_o SOOTY	*Ba* BAR
A. Black by black blue-tail									
168+	28B	*SsTtC(C)*	71A	*ssTtC(C)*	3	1	6		
1076+	841A	*ssTtC(C)*	799B	*SsT(T)*	1	1			
B. Black blue-tail by black blue-tail									
1592	1457E	*ssTtC(C)*	1457N	*ssTtC(C)*	(3)	2	2		
C. Black blue-tail by check									
808+	194B	*ssTtC(C)*	559A	*ssttC(C)*			4		
1610	1457Q	*ssTtC(C)*	837B	*ssttC(C)*			2		
1694	1533V	*ssTtCc*	1607W	*ssttCc*			3		1
D. Black blue-tail by bar									
1596	1533K	*ssTtCC*	1448E	*ssttcc*			6		
1608	1533V	*ssTtCc*	1472Q	*ssttcc*			12		
1743	1533K	*ssTtCC*	$1443C_2$	*ssttcc*		2	12		
1744	1533V	*ssTtCc*	1454M	*ssttcc*		1	3		
E. Black by bar throwing sooty									
1472	829B	$Ss(TT)$ CcS_os_o	1296N	$ssttccs_os_o$	5		1	3	2

MATING	♂♂	COMPOSITION	♀♀	COMPOSITION	S BLACK	T BLACK BLUE-TAIL	C CHECK	S_0 SOOTY	B_a BAR
F. Check by bar throwing sooty									
30+	26A	$ssttccs_0s_0$	27A	$ssttCcS_0s_0$			6	1	8
1453	1077A	$ssttccs_0s_0$	1196A	$ssttCcS_0S_0$				1	2
1652	1526H	$ssttCcS_0s_0$	1443C^2	$ssttccs_0s_0$			4	2	1
1802	1704E	$ssttCcS_0s_0$	1788A	$ssttccs_0s_0$			3	1	2
Total (4 matings)							13	5	13
Expected (2:1:1 ratio)							15.5	7.75	7.75
G. Check by sooty									
1342	1091J	$ssCc(S_0)$	1173A	$ssccS_0(s_0)$				1	
1765	1708B	$ssccS_0(s_0)$	1607B$_2$	$ssCc(S_0)$			6	5	1
1760	1657T	$ssCc(S_0)$	1652B	$ssccS_0s_0$			1	1	1
1759	1638D$_2$	$ssC(CS_0)$	1173A	$sscc(S_0)$			1		
H. Sooty by sooty									
1482	1385A	$ssccS_0s_0$	1173A	$ssccS_0s_0$				2	2
I. Sooty by bar									
1664	1462H	$ssttccs_0s_0$	1491D	$ssttccS_0s_0$				3	4
1798	1777A	$ssttccS_0s_0$	1665B	$ssttccs_0s_0$				1	2
1669	1568U	$ssttccS_0s_0$	1668A	$ssttccs_0s_0$				1	4
Total (3 matings)								5	10

not carry S and hence should not produce blacks. It so happens, however, that the preponderance of the data presented in substantiation of the theory on the genetic relations of the various blue types to black are taken from matings of Homers and Tumblers, while these three exceptions occurred in a single mating of a pair of Fantails. It seems possible, therefore, that this breed may carry some modifying factors which disturb the usual relations.

Such a presumption is further substantiated when the several matings of black blue-tailed Tumblers and Homers to checks and bars of the same breeds (sections C and D) are considered. In seven such matings 46 offspring were produced and none was full black. Full-black, then, may carry the factor for black blue-tail (T), but black blue-tails, when bred to bars or checks, do not (with the exception above) throw full-blacks, that is, they do not produce offspring with more black than they themselves show.

The typical check is the next stage lower in the series. The distinction between check and black blue-tail is not as readily made as between full-black and black blue-tail. It is often difficult to differentiate between a very slight check (figure 11C) and a black blue-tail, as some birds have only a very little checking in a few of the greater wing coverts. In this case the classification has to be arbitrary, and for present purposes those birds with any checking on the wing coverts are considered as checks.

The breeding behavior of check, as already intimated, is similar to that of black blue-tail. In eleven matings of typical checks to checks (see page 476) only checks were produced, with one doubtful exception in which two slight checks produced a black blue-tail.[5] The squab died young, however, and at this time showed two shades of black instead of black and blue. Had it lived to maturity it might have moulted to a typical check, the poor black becoming at least somewhat blued. In the twenty-one matings of checks to bars (page 477) no offspring with more black than that shown by the checked parents resulted.

The writer's data on the Mendelian behavior of the sooty character are meager, as they were for the black blue-tail pattern. Nevertheless, they illustrate the same point. Sections E and F of table 5 show that full-blacks and checks when bred to blues may throw sooties. The numbers obtained in section F are, so far as they go, in fair accord with the present hypothesis. The checked parents are Cc and carry the factor for sooty in a heterozygous condition (CcS_0s_0), for they throw sooties when bred to bars which lack both check and sooty (ccs_0s_0). In this case a 2:1:1 ratio is expected. There is a preponderance of blues, which, however, may be due to the fact that the early descriptions made for the birds of mating 30+ were not as detailed as were the later ones for the other matings, and consequently, some of the 8 barred birds might be sooties. Section G gives the results of breeding sooties to checks, in which case sooties and checks are recovered. When sooty is bred to sooty or to bar (sections H and I) only sooties and bars result, no checks or forms higher in the series than the sooty being obtained. A 1:1 ratio is expected from the matings given in section I. There is a decided preponderance of blues, which is attributed to small numbers.

Bar, the fifth in the present series, has already been discussed in detail. It is easily differentiated from sooty and check by a complete absence of any black on the wings except the black bars. This type, it may be remembered, bred true; it did not produce offspring with more black than shown by the two wing bars.

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[5] It is possible that the arbitrary point of demarkation referred to in the preceding paragraph may be genetically wrong and that these *slight* checks are really black blue-tails.

All that can be said at this time regarding the inheritance of barless is that it is apparently recessive to bar. A barred Homer crossed on a barless Homer gave four barred offspring and one which died before the wing bars developed, that is, before they normally develop in pure barred stock. In another mating the male was black but known to be heterozygous for S and to carry the factor for barring. The female was a barless Swallow. Only three offspring resulted, of which two were blue with black bars and one was a full-black with blacker bars. The data, though very few, nevertheless indicate that barlessness (b_a) is recessive to bars (B_a). No F_2 data have as yet been obtained, but there is every reason to believe that either complete segregation or at least a series which will show no more black than the blue black-barred grandparent, will result.

As has been frequently pointed out the blacker types throw individuals of various lower but not of higher grades. For instance, blacks may throw blacks, black blue-tails, checks, sooties and bars; black blue-tails (with one exception) throw black blue-tails, checks, sooties and bars; and so on, down the series.

Apparently, then, these six characters form an epistatic series illustrated in figure 7. This relation of several factors might naturally suggest multiple allelomorphs. They do not, however, fall into this category, since blacks known to be heterozygous for bar, when bred to bars, have produce checks (table 4, page 479), which is inconsistent with the theory of multiple allelomorphs. Furthermore, if these factors formed a multiple-allelomorph series no more than 3 types could ever be recovered from any one cross. On the epistatic-series interpretation it should undoubtedly be possible, however, to obtain the six types of the present series from suitable crosses, and 4 have actually resulted (table 5, section E) in a cross of a black by a bar.

So far as the data go the various factors are apparently not linked, but each is carried on a separate pair of chromosomes. Furthermore, these several factors do not have a cumulative effect as in quantitative inheritance, but each is capable of producing its particular effect in the absence of the ones higher in the series.

This series pertains to the orderly relation of various conditions of one character, these conditions being due to factors not allelomorphic to any of the other factors. Obviously, such an epistatic series cannot, as BREITENBECHER (1921, p. 66) suggests in his paper on multiple allelomorphs in Bruchus, also form a multiple-allelomorph series.

The epistatic series is of special interest in the light of WHITMAN'S selection experiments. He was able to select for fewer and fewer checks until he obtained a typical blue black-barred wing and finally one even without bars, but he was unable to reverse the process. These results led him to believe that the check pattern had probably evolved in an

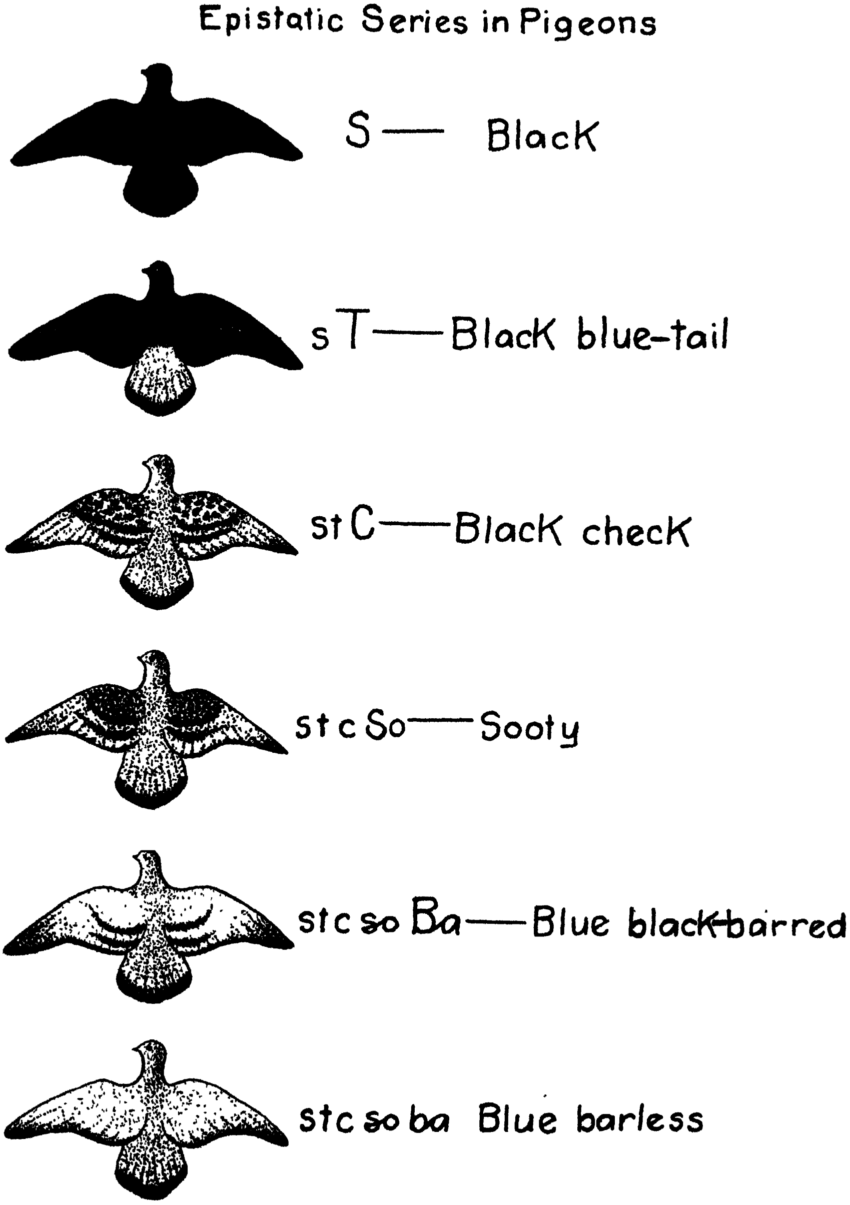

FIGURE 7.—Six distinguishable grades of the epistatic series showing the successive decrease in the amount of black (spread pigment) in relation to blue (clumped pigment).

orthogenetic fashion and that this type of evolution was continuing. His results, however, are quite in keeping with the genetic behavior of the factors in the epistatic series just discussed. Obviously mere heterozygosis is sufficient for obtaining the lower grades of check and bar, while it is very apparent that the higher grades can only result from dominant mutations. This would account for WHITMAN's failure to obtain offspring with much checking from parents with only a little.

If the wild Rock is the progenitor of the domestic races of pigeons it seems evident, then, that black and black blue-tail have occurred by dominant mutations.

Relation of the factors, S, T, C, and S_0, to other known heritable colors

COLE (1914) showed that the S factor could be carried by red and yellow individuals. In other words the presence of B, the factor producing black pigment,[6] was necessary for the manifestation of S. In a similar way the presence of B is necessary for C to act, but S as already pointed out, must be absent. The same is true of the T and S_0 factors. Seven matings in which reds were mated to bars produced blacks, checks, sooties and bars, obviously showing that these reds carried S, C and S_0. If more birds had been tested, no doubt some carrying T would have been found. Nine matings of yellow and bar proved that yellows also transmit these four factors.

The C factor is carried in a similar way by whites, as shown by various crosses made by STAPLES-BROWNE (1912) of bars on whites with the result that the offspring were frequently checked. There is no reason to suppose that others with S, T and S_0 do not also exist.

The six patterns discussed are distinguishable in the dilute series as in the intense. That is, parallel to the first five grades of the intense series there are full-duns, dun silver-tails, dun checks, sootied silvers and silvers with dun wing bars. By analogy there should also be silver barless. Difficulty was sometimes found, however, in differentiating these dilute types because of the variations in the shades of dun. Many full-duns, that is, S birds, may have darker terminal bands on their tails, which complicates the usual easy distinction between S, T and C birds in the intense series, since the tails of the latter are as a rule uniformly black without a blacker terminal band.[7] In questionable cases of the dilute series a microscopic study of that part of the tail anterior to the band, where clumped pigment is expected in checked and barred birds, was often necessary.

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[6] The action of B may perhaps better be considered as *extending* black pigment, making it analogous to E in mammals (see COLE and KELLEY 1919, footnote 4, p. 186).

[7] Two individuals of the SI type have been noted among the 1920 offspring, as having a darker terminal band with a lighter area just posterior to it. This area has somewhat of a bluish cast. When examined microscopically, however, it shows only clumped pigment as does the corresponding area in duns (Si).

The next consideration was to correlate, if possible, the grades of the epistatic series with the various shades of red and yellow produced by the sex-linked black-restricting *A* factor. This, however, proved impossible with the present knowledge of the many variations of color due to this factor. There exist the so-called red-barred and red-checked types, but it was sometimes found upon testing that these did not always correspond to what was meant by check and bar in the *a* series. It is plain that the only way to determine the corresponding patterns in the two series is to test the many varieties of the *A* series by breeding to *a* bars. Until such work has been done an attempt to distinguish the *A* epistatic series will be postponed. Preliminary experiments demonstrated that *A*-factor birds may carry S, C and S_o. If more individuals had been tested, some carrying T would no doubt have been isolated as well.

While, then, the factors which produce the various grades of the *a* epistatic series can act only in the presence of B and absence of S, they can be carried by b and S individuals, by whites and by restricted reds. In other words they may be present in any of the eight known heritable colors of pigeons, but can not show except under certain favorable factorial conditions.

UNIFORM AND LIGHT OUTER VANES OF OUTER TAIL FEATHERS

The variation in the color of the outer vanes of outer tail feathers represents another modification of blue and black. Anyone familiar with pigeons is acquainted with the so-called light outer-vane condition found in both blue- and black-tailed birds (figures 12 and 2). It is restricted to that part of the outer vanes of the outer tail feathers proximal to the region occupied by the black terminal band characteristic of blue-tailed birds. This entire region or only the outer edge may be affected. DARWIN and LYELL call attention to it in descriptions of various breeds of pigeons, especially the wild Rock. The contrasting condition, uniformity, refers to the fact that this portion of the outer vane is similar in color to the other tail feathers, that is, in S or black-tailed birds it is black (figures 1 and 8A) while in blue-tailed or s birds it is blue (figures 9 and 8C).

A microscopic examination of the two conditions, uniform outer vane and light outer vane, in S birds (full-black, with black tails) shows them to be different. The pigment in the former (figure 8A) is spread, while in the latter (figure 8B) it is clumped. The uniform outer vanes of blue-tailed individuals (figure 8C), however, show clumped pigment. This is to be expected, since the factor *s* has already clumped the pigment and, as mentioned above, uniformity merely means that the outer vanes shall

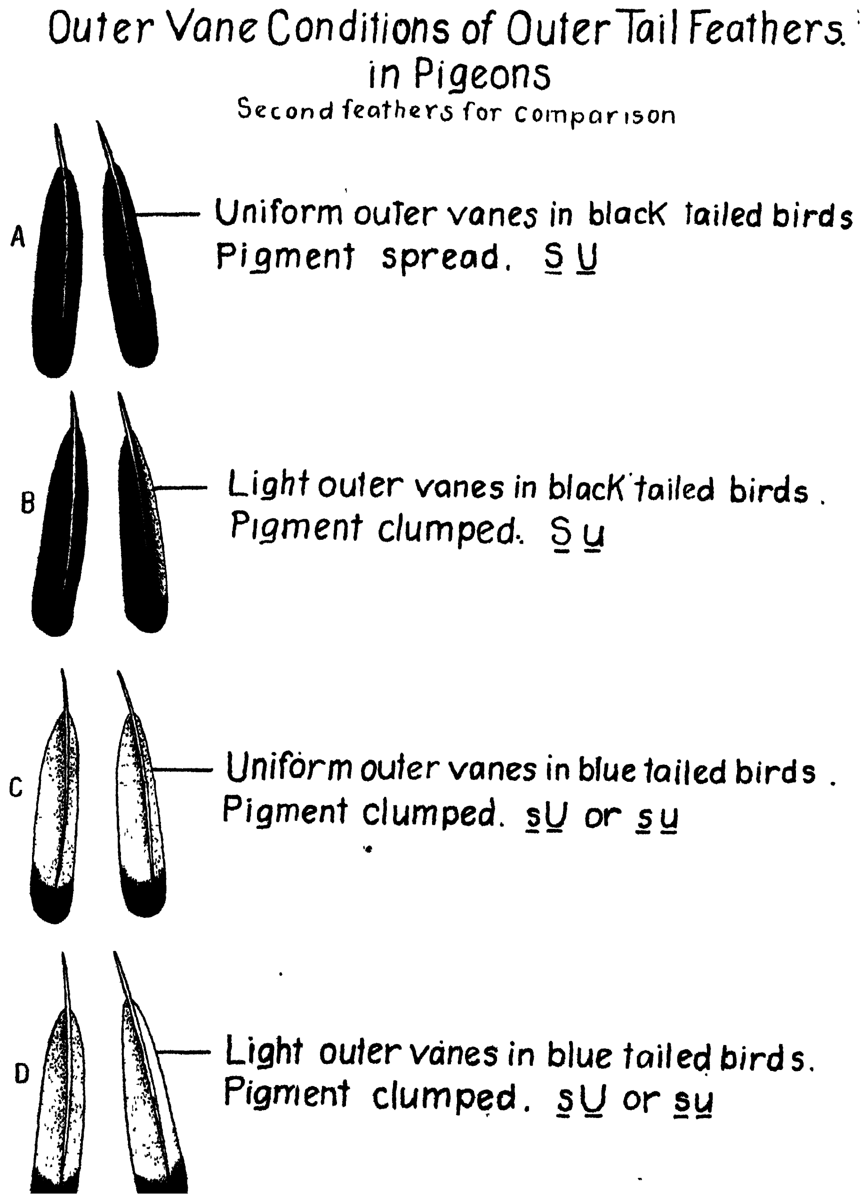
Outer Vane Conditions of Outer Tail Feathers in Pigeons
Second feathers for comparison
A
Uniform outer vanes in black tailed birds
Pigment spread. S U
B
Light outer vanes in black tailed birds.
Pigment clumped. S u
C
Uniform outer vanes in blue tailed birds.
Pigment clumped. sU or su
D
Light outer vanes in blue tailed birds.
Pigment clumped. sU or su

FIGURE 8.—Uniform and light outer vanes in *S* and *s* birds. Another tail feather is shown in each case for comparison.

correspond to the other tail feathers. The light condition (figure 8D) of the outer vanes of blue-tailed birds appears to be due to a diminution in the amount of pigment. The difference, then, between light and uniform outer vanes in *S* birds is due to clumped and spread pigment, while an apparently corresponding condition in *s* individuals depends upon the amount of pigment, which is clumped in both cases.

The foregoing may be explained on the assumption that a factor *U* spreads black pigment in the outer vanes of outer tail feathers of *S* birds, while *u* clumps it. Since *s* has already produced clumping in the tail region of blue-tailed birds the effect of *u* is not noticeable, and apparently *U* can not act in the presence of *s* Still there is no reason to believe that *s* birds can not carry *U*.

Figure 9.—Uniform outer vanes in a blue-tailed bird. (Wisconsin No. 1704F.)

Before taking up the data in substantiation of such a theory two points must first be considered. The first is the variation in the degrees of lightness exhibited by different individuals. This is due in both black and blue-tailed birds to differences in the amount of pigment. It may depend, also, upon the age of the birds, younger ones showing more pigment than older ones. In fact it was found from a study of the several descriptions of many individuals that the first frequently gave the outer vanes as "uniform," while later descriptions, made after the birds had moulted, often gave them as "light." This was true of both types of birds. As a consequence the left outer tail feathers were plucked from squabs of various ages to determine whether this, like a normal moult, would tend to decrease the amount of pigment in the incoming feathers.

Such proved to be the case, as illustrated in figure 10, which represents 2 and 3 plucks from twelve birds. In view of the foregoing it was necessary to use only old birds, or squabs from which one of the outer tail feathers had been plucked at least once, before compiling the tables which follow.

The second point for consideration is lightening due to factors other than *u*. In certain cases of black-and-white-mottled birds the outer vanes may be light but such pigment as is present is spread, which probably indicates the action of factors for white spotting. Consequently in any doubtful cases a microscopic examination was necessary. The factors for white spotting are not included in the consideration of light and uniform outer vanes, but only *U* and *u*.

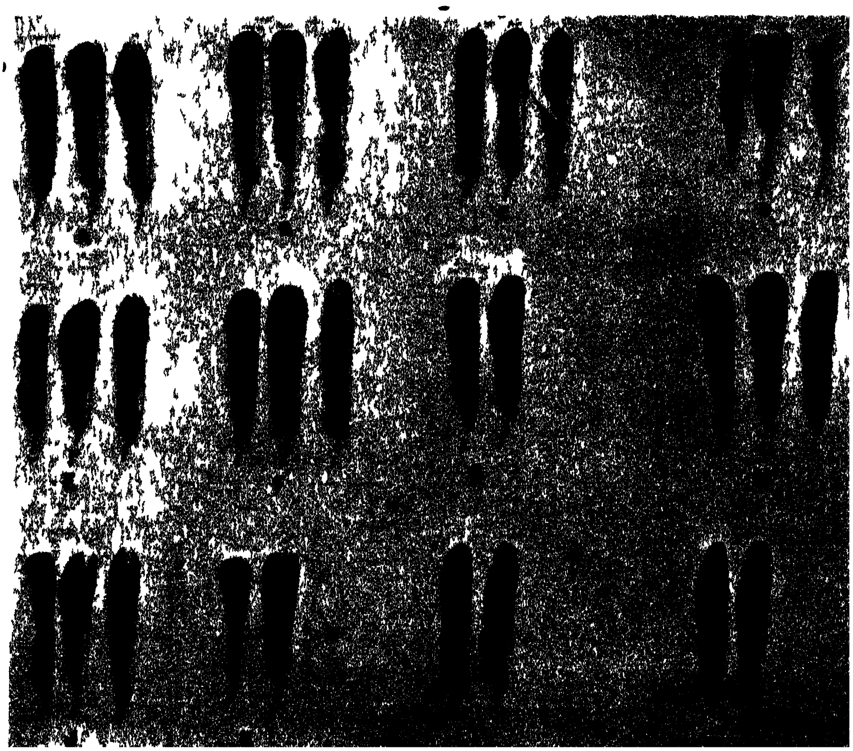

FIGURE 10.—Outer tail feathers of squabs, plucked successively upon their regrowth, showing the acquirement of light outer vanes in the later feathers.

The dominance of *UU* in the presence of *SS* was indicated by seven matings which produced thirty-six uniform-outer-vaned offspring. The homozygous condition of *U* was judged from the offspring. No mating producing less than five individuals was used except in one case where the pedigrees of the parents indicated their compositions. In the other matings some of the birds were "original" and consequently were unpedigreed, while others having pedigrees showed *b* or *s* or *A* birds in their immediate ancestry, making it impossible to judge of their composition.

Other matings showed the segregation of *Uu* in the presence of *SS*. Three matings in which both parents were *Uu* produced 18 "uniform" and 4 "light" black-tailed offspring, which is, considering the numbers, fairly close to expectation (16.5 : 5.5). Two matings of *Uu* to *uu*, from which a 1:1 ratio is expected, gave 10 : 11.

If *u* is a definite factor independent of *S*, then when *SSuu* or *Ssuu* birds are bred either *inter se* or to *s* individuals of the composition *uu*, all the *S* offspring should have light outer vanes. This condition has been only partially tested. An *Ssuu* (figure 8B) bird was bred to an *s* bird with light outer vanes (figure 8D), the condition of which with respect to *U* could not, of course, be phenotypically judged. From such a mating, however, nine offspring resulted, five *S* and four *s*, all of which had light outer vanes. Three of the *Ssuu* offspring were in turn mated to light-outer-vaned *s* birds and produced 26 offspring. Fourteen of these were blacks (*S*), and all, with the exception of one which died young before the first-plucked outer tail feathers had been replaced, had light outer vanes. This showed fairly conclusively that the original bird tested was of the composition *Ssuu*. Furthermore, the *s* bird to which it was mated, also evidently lacked *U*, as did the *s* birds to which the three *Ssuu* offspring of the original pair were bred.

When, however, two *Ssuu* (figure 8B) were bred together they produced an *SU* (figure 8A) individual, which is a decided exception and no explanation for which is at present apparent. This is a weak point in the theory, for only *u* birds should have resulted. It is recognized that the data which are presented in substantiation of the foregoing hypothesis are few, and in order to further strengthen it more *SSuu* and *Ssuu* birds should be thus tested. In this event another theory might better explain the data, but with the present records the present hypothesis seems to be the most satisfactory one available.

If, as supposed, *U* is unable to act in the presence of *s*, any definite breeding behavior of so-called light- and uniform-outer-vaned *s* birds is not expected. Of 51 matings of phenotypically light-outer-vaned blue-tailed birds bred *inter se*, four threw some offspring which had uniform outer vanes (figure 8C). As already mentioned, however, the point of difference is amount of pigment, so naturally the *U* factor just postulated would have no apparent effect. These four matings refer only to those in which offspring were of a sufficient age to have passed through at least two or three moults or have had their outer tail feathers plucked several times. Seven other matings also show the production of uniform-outer-vaned offspring, but these individuals died young, and hence are not considered in this connection for reasons previously mentioned.

To show further that so-called uniformity in *s* birds is not the same as in *S* individuals, two so-called uniform-outer-vaned blue-tailed birds were bred to light ones and only light-outer-vaned offspring (16 in number) resulted. This plainly indicates that the genetic relation of the light and uniform conditions in *s* birds is complicated.

TABLE 6

Matings of SsUu birds × Ssuu birds.

MATING	♂♂	COLOR	COMPOSITION	♀♀	COLOR	COMPOSITION	OBSERVED			EXPECTED		
							SU	*Su*	*sU*+*su*	*SU*	*Su*	*sU*+*su*
554+	450X	Dun	*Ssuu*	450Y	Dun	*SsUu*	5	1	1	2.54	2.54	1.76
662+	493B	Black	*Ssuu*	454B	Black	*SsUu*	4	7	1	4.04	4.04	2.76
1047+	829B	Dun	*SsUu*	829A	Dun	*Ssuu*	1	1	1	1.14	1.14	.76
1024+	901B	Black	*SsUu*	890B	Dun	*Ssuu*	(1*)	1	1	.75	.75	.50
Total (4 matings)							10	10	4	9	9	6

* Died young, therefore omitted from consideration.

Table 6 gives matings of *S* and *s* birds from which a 3:3:2 ratio is expected. The numbers are small (10:10:4) but approach the calculated (9:9:6) rather closely. Table 7 shows thirteen matings from which if the *s* birds are *Uu* a 3:1:4 ratio is expected and if they are *uu* a 1:1:2. They are known from pedigree and offspring to be of one or other of these compositions. The closeness of fit (P) for the first of these ratios is poor but for the second, good. There are, however, doubtless both *U* and *u* birds among those which are *s*. If it is assumed that half are *U* and half *u*, the expected ratio would be 5:3:8 (3:1:4+2:2:4). The closeness of fit on this assumption is excellent, χ^2 being less than 1. The numbers are, to be sure, small and consequently not too much importance is to be placed upon them, but the ratio at least lies between 3:1:4 and 1:1:2. It may, therefore, be assumed that the *s* parents comprised both *Uu* and *uu* individuals.

In order to prove further that *s* birds carry *U*, blue-tailed individuals with phenotypically light outer vanes have been bred to *Ssuu* individuals, n which case any *SU* offspring must have gotten *U* from its *s* parent.

TABLE 7

Matings of SsUu birds × ssUu or ssuu birds.

MATING	♂♂	COLOR	COMPOSITION	♀♀	COLOR	COMPOSITION	OFFSPRING		
							SU	Su	$sU+su$
826+	6A	Black	$SsUu$	639B	Silver	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	1	1	4
837+	639A	Silver	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	719B	Black	$SsUu$	2	1	2
1448	1341D	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	915B	Dun	$SsUu$	7	1	3
1454	1385B	Black	$SsUu$	1242A	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	5	1	3
1472	829B	Dun	$SsUu$	1296N	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	3	2	5
1605	1341D	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	1500H	Dun	$SsUu$	1	1	4
1681	1512R	Black	$SsUu$	1657Z	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	......	1	3
1682	1448T	Black	$SsUu$	1668A	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	......	2	7
1693	$1593C_2$	Black	$SsUu$	$1607B_2$	Check	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	......	1	1
1695	1568B	Black	$SsUu$	1607U	Check	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	1	1	1
1745	1638A	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	1484A	Black	$SsUu$	.....	1	1
1761	1455V	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	1553B	Black	$SsUu$	2	2	1
1773	1568B	Black	$SsUu$	1296N	Blue	$ss\left\{\begin{matrix}Uu\\uu\end{matrix}\right\}$	2	1	3
Total (13 matings)							24	16	38

Expected:

1. If *s* birds are *Uu*, a 3:1:4 ratio is expected.............	28.89	9.63'	38.52
2. If *s* birds are *uu* a 1:1:2 ratio is expected..............	19.50	19.50	39.00
3. If one-half of the *s* birds are *Uu* and the other half *uu*, a 5:3:8 ratio is expected..........................	23.35	14.61	38.96

1. $\chi^2 = 5.0443$ P = .0806
2. $\chi^2 = 1.178$ P = .5620
3. $\chi^2 = .173$·

Of some twelve birds tested seven have been found to be *SU*, which upholds the above presumption. There are two matings on which data are lacking because in each an *S* squab died before the replacement of the first plucked feather.

Evidently there is a decided interaction of S, U, and their allelomorphs. S permits of the phenotypic expression of both U and u, while U can not express itself in the presence of s. Since both u and s produce the same effect, namely a bluing due to clumped pigment, they are indistinguishable when combined. No definite factor was found for the difference in amount of pigment in the outer vanes of s birds.

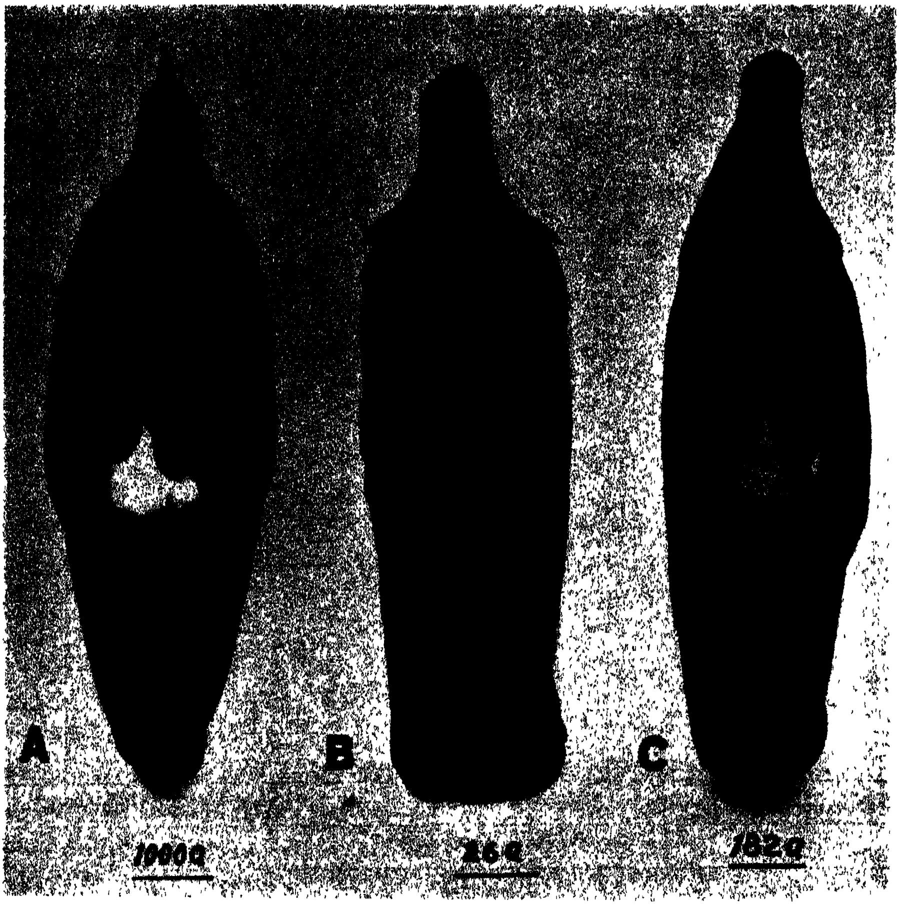

FIGURE 11.—A. Extreme light rump (grade 1) in blue black-barred wild Rock pigeon. (Wisconsin No. 1000A.) B. Uniform blue rump (grade 3) in blue black-barred common pigeon. (Wisconsin No. 26A.) C. Very slight check with light blue rump (grade 2). (Wisconsin No. 182A.)

LIGHT AND UNIFORM RUMP

The two rump colors "white" and blue, found respectively in *C. livia*, particularly common in Europe, and *C. intermedia* of India, have long attracted the attention of naturalists. This is the one mark of differentiation between these two species. According to SALVADORI (1893, p. 255), however, some specimens of *C. livia* may have the blue rump, in which cases they are indistinguishable from *C. intermedia*. In fact SALVADORI (p. 256) states that "every ornithologist knows the difficulty of drawing the exact line between *C. livia* and *C. intermedia*." This question is discussed in some detail by DARWIN (1868) and others already cited. The former attributes the difference in color of these two types to climate, the warmer environment of India tending to produce a color uniform with the rest of the bird.

"White" and blue rumps (figures 11A and 11B), together with many intermediate shades, also exist in barred and checked birds of several varieties of domestic pigeons. A detailed examination of many birds of the intense series admits of three fairly definite categories. Only intense individuals were used, as those of the dilute series presented another, but presumably strictly parallel, range of much lighter shades which were more difficult to classify.

The first or lightest grade is the "white" rump, typical of *C. livia*. This is preferably termed "extreme light blue" because a microscopic examination shows the presence of a small amount of clumped pigment, while white implies the absence of any pigment. The first grade is indicated below as number 1.

s
Blue-tailed

1. Rump extreme light blue—typical of *C. livia* (figure 11A).
2. Rump light blue—several shades of light blue (figures 5 and 6).
3. Rump uniform blue—typical of *C. intermedia* (figure 11B).

S
Black-tailed

4. Rump dark blue—found in some black birds (figure 12).
5. Rump black—uniform with the rest of the plumage (figure 1).

The next grade (number 2), found very frequently in general stocks of pigeons, is light blue. This color is not suggestive of white but of a silvery blue. Furthermore, the range of shades in this class is rather wide, extending from a very light blue, distinct from grade 1, to a shade just perceptibly lighter than uniform blue, grade 3. Uniform blue, as suggested in the first paragraph, refers to the correspondence in shade between rump, back and wing coverts. The pigment is clumped in grades 1, 2 and 3. The amount of pigment is apparently the determiner of the various shades, the lighter ones having less pigment than the darker. There are, then, at least three color-grades of rump in the *s* or blue-tailed birds, depending upon the amount of clumped pigment.

A consideration of rump conditions in black-tailed birds reveals two fairly definite types. Certain *S* individuals may be solid black except for a lighter shade of rump, the feathers of which give a dark blue appearance, (grade 4). It is a darker blue than any above described. The pigment, however, is clumped as it was in each of the foregoing cases. This grade, like the light blue (2), shows some range in shade, but for present purposes these shades will all be considered together under 4.

The last type (grade 5) occurs in *S* individuals and refers to a black rump uniform with the rest of the black plumage. In this case the pigment is spread.

These conditions are analogous to those found in the outer vanes of outer tail feathers of both blue- and black-tailed birds. In both cases the pigment is clumped in *s* birds and the shade depends upon the amount of pigment present. The dark blue rump of black birds corresponds to the light condition in the outer vanes of *S* birds in that the pigment is clumped, while the black rump and uniform outer vanes both have spread pigment.

The genetic analysis of the rump conditions is, however, more difficult than that of the outer vanes. The work of STAPLES-BROWNE (1908, 1912) is, so far as the writer is aware, the only report on the definite inheritance of these characters. His records are based on breeding tests of *s* birds only. Extreme-light-blue rump (1) appears to be dominant to uniform-blue (3), to segregate fairly cleanly, except for the appearance of some light blues (2), and uniform blue breeds true. He classes the light blues (2) with the uniform blues (3). WHITMAN (1919, vol. 2, table 87c, p. 112) obtained a uniform-rumped individual from a pair of presumably extreme-light-blue-rumped (1) wild *C. livia.*

The writer has collected considerable data from many matings and in certain cases obtained results corresponding to those of STAPLES-BROWNE. When, for instance, extreme-light-blue-rumped (1) birds were bred to light-(2) or uniform-blue-rumped (3) ones, all three types resulted. From fourteen matings of uniform-blue rumps (3), bred *inter se*, types 2 and 3 resulted, while one such mating also produced grade 1. This latter case is not in keeping with STAPLES-BROWNE'S findings. The writer's data correspond to those of STAPLES-BROWNE in that types 1 and 3 do not segregate cleanly when crossed, but produce light blue rumps (2). As previously stated STAPLES-BROWNE considered these latter as uniform blues (3). It has been found, however, that the light shades, when bred together or to uniforms, throw extreme-light-blue-rumped (1) offspring as well as uniform (3)- and light-blue (2)-rumped individuals.

In view of these discrepancies the writer made another classification for all matings,[8] including types 1 and 2 under one head and type 3 under the other, whereas STAPLES-BROWNE made one class of type 1 and combined 2 and 3. On such a basis as the former, that is, making a single class of 1 and 2, and one of 3, results are equally confusing. When extreme (1) or light (2) blues heterozygous for (3) are bred together, all three types result in a proportion of 3 : 1 (37 : 13), types 1 and 2 being combined to form the larger class. If heterozygotes, grades 1 and 2, carrying 3, as indicated by pedigree, are crossed on grade 3, the assumed recessive, the results of thirteen matings show 30:54 in the respective classes, that is, twice as many recessives as dominants. Type 3 does not breed true, but produces as many extreme and light blues (1 and 2) as uniform (3), the numbers being 37:39 from 14 matings. Obviously, then, this scheme comes no nearer to explaining the inheritance of rump conditions in *s* birds than did that of STAPLES-BROWNE.

Further instabilities are encountered when the breeding behavior of the dark blue (4) rump of black birds is considered. As already mentioned STAPLES-BROWNE'S data related to *s* birds only. Dark-blue-rumped (4) *S* offspring may be obtained from light(2)- or extreme-light(1)-blue-rumped birds, crossed on black-rumped (5) blacks. When these dark blues (4) are mated together they may produce a very small proportion of individuals like themselves (1 in 21 offspring), and they may throw some light (2) *s* offspring. Three matings of type 4 by type 2 produced all five types. Similarly, when grade 5 is crossed on grade 3 (24 matings) the five types result. If, on the other hand, black rumps (5) are mated to black rumps (17 matings) an occasional grade 2 may appear, but the majority belong to grade 5.

Evidently the inheritance of the various rump conditions in blue- and black-tailed birds is due to a complicated interaction of factors. In order to obtain the correct genetic analysis more detailed breeding experiments must be undertaken.

THE RELATION OF THE DULL-BLACK CHECK TO DEEP AND DULL BLACK

The modifications of black dealt with so far have been due to the relative amounts of the so-called colors, blue and black, exhibited by certain individuals, or in other words to the encroachment of clumped pigment upon spread. There is another diversity of black of another sort in that it does not involve bluing but is a modification of the depth and sheen of the color of many black birds. Some individuals may have either a brownish or a powdery plumbeous cast instead of a deep uniform velvety one. These conditions are considered undesirable by breeders of black varieties of pigeons. CHAPMAN (1911) calls attention to them as "very grave defects."

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[8] Only those birds for which rump feathers have been saved or the descriptions record "Rump lighter than wing coverts," or "Rump same shade as wing coverts," have been used in these tables.

Furthermore, some of these birds may show darker indistinct wing bars in the usual position (figure 13) or in some cases check marks lighter than the general color in the wing coverts (figure 12). These are due to two shades of black instead of the usual blue and black of the epistatic series described in earlier sections of this paper. BOITARD and CORBIÉ (1824) mention the cropping out of indistinct wing bars on black birds in certain crosses.

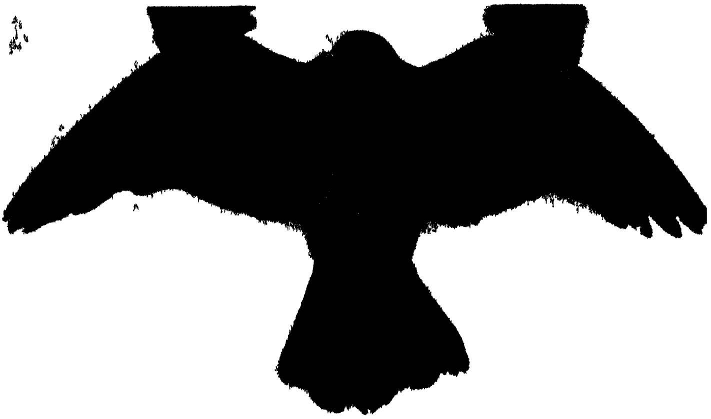

FIGURE 12.—Dull black check with dark blue rump (grade 4) and light outer vanes of outer tail feathers. (Wisconsin No. 1827C.)

Checks and bars of this description are likewise prevalent among duns (dilute blacks). The two main differentiating points between checks of this type, that is, dull-black or dun, and the blue type, are, first, the absence of the typical blue or silver tail with a black or dun terminal band, and second, the production of checks and bars by two shades of black or dun, and not by black and blue or dun and silver. In other words these checks and bars carry *S* while the blue type does not. These two points are further emphasized when the pigment-granule arrangement is studied, for all pigment in the dull-black or dun checked birds is in a spread condition. It is, however, more dense in the wing bars and darker areas surrounding the check marks than in the checks themselves and regions about the wing bars. The deeper-colored areas correspond to the black of barred and checked birds of the blue-black epistatic series, while the lighter areas correspond to the blue color.

Unfortunately breeding data on these patterns are very few. There are, however, several matings which give the breeding behavior of dull black (*d*) without bars or checks as contrasted to deep black (*D*). The chief distinguishing characteristics between this factor and *I* which affects density are (1) *D* is not like *I* in being sex-linked, (2) the difference in shade between *d* and *i* birds is readily distinguishable and (3) so far as is known at present, *d* individuals are indistinguishable from *D* in the presence of *i*.

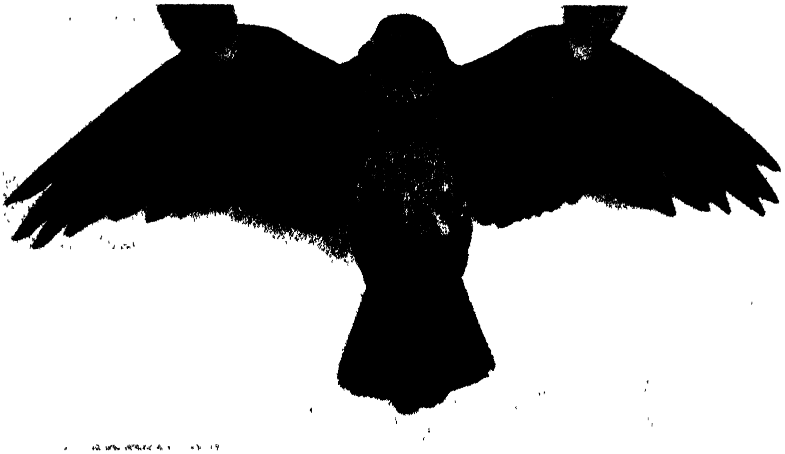

FIGURE 13.—Dull black with blacker bars, uniform rump (grade 5) and light outer vanes of outer tail feathers. The light appearance of the rump is not due to bluing but to a very dull black. (Wisconsin No. 1761J.)

The data on the relation of *d* and *D* in blacks are presented, because, to anticipate a little, it seems evident that the *d* condition is necessary for the manifestation of the two patterns. *D* does not permit of the presence of any contrasting shade or color, for birds carrying it are deep blacks uniformly pigmented.

This character, like that of the outer vanes of the outer tail feathers, changes with age. Most if not all black squabs are dull, and not until they have moulted at least once does the deep plumage, if they are to have that as adults, appear. Accordingly only adults are considered in the following discussion.

Certain black Baldheads and self-black Tumblers that have been used in this work have had the desirable deep-black feathering. Two matings of such black Baldheads produced four offspring deep-black like their parents, indicating that one or both of the parents were homozygous for *D*. Three matings of "original" deep-black Tumblers bred to deep-black checks gave a different result. "Checks" in this connection refers to the blue or *sC* check, in which the black can be either dull or deep. Since the Tumblers were original birds their genotypic compositions were unknown. The checked parents were, however, from colony stock and known to be *Dd*. As both deep and dull blacks were produced it was concluded that the Tumblers were likewise heterozygous. There were 12 of the former and 8 of the latter, where a 3:1 ratio was expected.

As just mentioned, the checks employed here refer to the *sC* of the epistatic series. Since such individuals present both blue and black, obviously the shade of black can be determined providing the black area of the wings is large enough, that is, if it occupies as much space on the wings as does the blue. A fairly large black region is necessary in order to admit of comparison. This was further emphasized when an attempt was made to compile data on the shade of black from bars and extreme checks. This was found impossible. As a consequence only blacks, black blue-rumps and slight to medium checks are used in these tables.

Table 8A shows six matings of heterozygous deep blacks bred to dull blacks, giving 14 deep to 11 dull blacks where the expected is 12.5:12.5. Section B shows the same thing except that some of the parents are *sC* checks. In this case the results are 14:16 and the expected is 15:15. A total for the 14 matings gives 28 to 27 where equality is anticipated.

Thirteen matings of dull to dull demonstrated the true-breeding behavior of this character, forty-four dull and no deep offspring being obtained. Obviously, then, the difference between dull and deep black is a single factor.

As previously mentioned, it is fairly apparent that *D* birds can not display the dull-black check and bars, because this would necessitate the introduction of poor black in deep-black individuals, which very evidently is not in keeping with the deep-black character. If this is the situation the present type of check, like the blue of the epistatic series, is due to an interaction of factors. That is, *d* is necessary for its manifestation as *s* is for that of the blue check, but the former carries *S* while the blue type lacks *S*. In the former respect the *s* and *d* conditions are analogous. The two checks may be termed the *sC*, typical of *C. livia*, and the *dS*, that just described.

TABLE 8

*Deep black (Dd) by dull black (dd).**

MATING	♂♂	COLOR	COMPOSITION	♀♀	COLOR	COMPOSITION	OFFSPRING	
							D	*d*
A. Blacks by blacks								
120+	45B	Deep black	*Dd*	56A	Dull black	*dd*	4	1
575+	448A	Deep black	*Dd*	432B	Dull black	*dd*	1	1
835+	719A	Dull black	*dd*	727A	Deep black	*Dd*	3	7
1317	1015B	Deep black	*Dd*	1127B	Dull black	*dd*	2	
1812	1685C	Deep black	*Dd*	$1594Q_8$	Dull black	*dd*	4	
1826	$1574F_2$	Deep black	*Dd*	1594R	Dull black	*dd*		2
Total (6 matings)							14	11
Expected							*12.5*	*12.5*
B. Black by check and check by check								
168+	28B	Dull black	*dd*	71A	Deep black check	*Dd*	4	5
1153+	972A	Deep black check	*Dd*	559A	Dull black check	*dd*	1	1
1346	972A	Deep black check	*Dd*	1033H	Dull black	*dd*		1
1684	1665A	Deep black check	*Dd*	1677A	Dull black	*dd*		1
1692	$1553J_2$	Dull black	*dd*	1616X	Deep black check	*Dd*	4	3
1693	$1593C_2$	Deep black	*Dd*	$1607B_2$	Dull black check	*dd*	1	
1695	1568B	Deep black	*Dd*	1607U	Dull black check	*dd*	1	1
1699	1657K	Deep black check	*Dd*	1605V	Dull black	*dd*	3	4
Total (8 matings)							14	16
Expected							*15*	*15*
Total for 14 matings							28	27
Expected							*27.5*	*27.5*

* Only adult birds considered.

CHECK MARKINGS IN THE COLUMBIDAE

The preceding evidence leads to the conclusion that the domestic pigeon presents two types of checks and bars, due to the interaction of at least three different factors, and varying in pigment-granule arrangement and density of pigmentation. It was pointed out in the introduction that there were several species closely related to C. *livia* which resembled the barred form and one or two the checked. These also correspond to the same patterns in the domesticated pigeon. The dull-black check described in the preceding section for domesticated varieties is a regular marking in C. *guinea*, closely related to C. *livia*. The check marks, which are V-shaped and situated at the tips of the wing coverts, are lighter than the surrounding areas, but the pigment is, in general, spread in all parts of the feathers. There is, however, a tendency to clump near the tips of the barbules, especially in the region of the V-shaped check mark.

These two types are found only in those forms closely related to C. *livia*. But there is another check condition which obtains in certain species outside the Columba group. In these the check marks instead of being lighter than the surrounding areas, as is usual in both the blue and dull-black checks, are darker than the rest of the plumage. Examples of this type are to be found in the mourning dove (*Zenaidura macroura carolinensis*) and the European turtle dove (*Turtur turtur*). In the former the few checks are located in the region of the usual wing bars of C. *livia*, and are black against the surrounding brown plumage. The dark checks of the turtle dove occupy the entire centers of the wing coverts, which are edged with a lighter shade.

Sufficient breeding data relating to the inheritance of this type of check are lacking. Microscopic examinations have been made, however, of the feathers of several individuals representing various species chosen at random. In all cases the pigment was spread throughout the feathers but was more dense in the dark check marks than in the lighter surrounding areas. This is just the reverse of the dull-black check, in which case the check is lighter than the rest of the feather, being the area of lesser pigmentation. It will be noted that in both cases all the pigment is spread, the differentiation of the pattern being based on density of pigment and not on arrangement of granules. The forms in which this condition was studied were *Zenaidura macroura carolinensis*, *Turtur turtur*, *Turtur lugens*, *Columbigallina rufipennis*, *Geopelia striata* and *Oena capensis*.

GENERAL DISCUSSION

The work by COLE (1914) and the present work on blue and black color in pigeons deal with two types of interacting factors. Both are dependent upon B[9] for their phenotypic expressions. All the factors of the first group, belonging to the epistatic series, produce similar effects, namely, clumping and spreading pigment. Each, however, is limited in its action to a particular region. For example, the factor for barring, B_a, spreads pigment in the wing-bar region, that for sooty, S_o, introduces a sprinkling of black color in the wing coverts, which may acquire definite black spots resulting in a checked appearance when *C*, the checking factor, is added. In this case the sooty areas become distinctly black by the addition of more spread pigment. Similarly, the effect of the checking factor is concealed by the full spreading of black to the wing coverts leaving either a blue rump and tail or only a blue tail, when *T* is present. *T* in turn is hidden by *S*, which spreads pigment throughout the plumage of the individual, making it a solid black. The epistatic series, then, consists of a set of factors each of which increases the area of spread pigment over that of its predecessor. Or, in other words, the respective dropping out of each of the several factors is necessary for the phenotypic expression of those lower in the series.

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[9] See footnote 6, p. 486.

The factors of the second group, namely, those for uniform and light outer vanes of outer tail feathers, uniform and light rump, and deep and dull black, are dependent upon *B* and *S* for their various expressions. They do not, however, lie in the epistatic series but interact differently. *U*, for uniform outer vanes of outer tail feathers, can express itself only in the presence of *S*, being indistinguishable in all *s* birds.

The several rump conditions vary somewhat similarly in black- and blue-tailed birds. Dark blue rumps are known to exist in *S* birds. They might, however, be found in the extreme type of black blue-tails (*sT*) in which ordinarily the tail is blue and the rump black like the wing coverts. This is pure supposition, as no birds of this description have been identified. Below these the rump colors change to those described for *s* birds.

Both *I* and the higher stages of the epistatic series, *S*, *T*, and some grades of *C*, are necessary for deep and dull black to make themselves apparent.

There are, then, two types of interacting factors, those of the epistatic series behaving in an orderly serial fashion, and others which are dependent upon certain stages of the series for their various expressions.

With the isolation of the several factors of the epistatic series comes the fact that the previously postulated ratios between *S* and *s* birds are changed. For example, it was pointed out above (p. 480) that COLE (1914) considered all checks (only checks were involved in the three illustrative matings used by him, no black blue-tails or sooties) as blacks and consequently he expected three blacks and checks to one barred. Since, however, the existence of the checking factor has been determined, the former 3: 1 ratio becomes a 15: 1, as two factors instead of one are involved. If, on the other hand, the present interpretation is correct, blacks heterozygous for S when bred *inter se* should give a 3:1 ratio, considering black blue-tailed birds and all individuals below them in the series as s. The combined available data fit this ratio well, with the observed numbers 71:28 and the expected 74.25 : 24.75. The probable error (P.E.) of the ratio is .12 and the $\frac{\text{Dev.}}{\text{P.E.}}$ is 1.08. Similarly, in the data giving an expected 1:1 ratio the total observed were 43:55 and the expected 49:49. The P.E. in this case is .068 and the $\frac{\text{Dev.}}{\text{P.E.}}$ is 1.47. These results are in keeping with the present hypothesis.

Unless some stage shall be found that is dependent upon S, the ratio between S and s will continue to remain the same. If, however, more intermediates involving both S and s should later be isolated, the various ratios existing between the present known factors will become altered. It seems, furthermore, highly probable that more factors are concerned, since the series appears to grade from black through to a pure blue wing lacking any black except that confined to the tips of the primaries, secondaries and tertiaries. If six grades in this series have been shown to exist independently, there is no reason why others could not also be split off, for students of Drosophila have found seven modifiers acting on one eye color alone, eosin, which in its turn lies in the graded multiple-allelomorph series from red to white.

As the members of the epistatic series increase, the chances for the occurrence of the recessives, especially those lower in the series, as the barred type, decrease. In fact, with the series as it now is, sooties and bars should appear rather infrequently if all the intermediate factors were involved. This, however, is not the case, but in several instances the number of barred birds, especially, is high. This is due, no doubt, to the fact that most of the birds making up the parents of the various matings are not heterozygous for all the characters under consideration, but for only one or two. In such a case more recessives of these types, are expected.

That the check series seems to grade almost imperceptibly from one type to another and is epistatic, is quite in keeping with WHITMAN'S selection experiments. He was able to select for less check but not for more. These results led him to believe that the evolution of checks and bars was an indistinguishable and gradual one moving in one direction. It does not, however, necessarily follow that because the interaction of these several factors produces an apparent epistatic series, the mutations producing the various grades of spread pigment occurred in any particular order. This is made even more unlikely by the fact that these factors are apparently independent and hence exist at different places in the germplasm.

The isolation of some of these factors, as particularly that for check, and a study of the various rump conditions are of interest not only from the evolutionary standpoint but also from the systematic. Certain characters governed by these factors have been used as marks of distinction between species. As pointed out earlier, SALVADORI admits that rump color is the only tangible difference between *C. livia* and *C. intermedia*. In the case of checks and bars in the wild Rock, DARWIN suggested that these two forms should be considered as a single species. He even went further and stated that several other species as *C. rupestris*, *C. schimperi* and *C. gymnocyclus* should also be included with *C. livia*. In view of the present knowledge of Mendelism, DARWIN'S suggestions are even more valid, since in certain of these cases at least, it seems evident that the species differences are based on a single factor. WHITMAN'S classification of *C. affinis*, which is checked, and *C. livia*, which is barred, illustrates this point.

Here the difference is presumably due to a single factor, *C*. If such marks are used for species differentiation, then the color variations of each variety of the various breeds of pigeons might as legitimately be assigned species names.

It seems possible, therefore, in view of the foregoing and the close resemblance in descriptions given by SALVADORI, of the species closely related to *C. livia*, that the domesticated pigeon may be, as suggested by GHIGI (1908), of polyphyletic origin and not descended from a single species, *C. livia*.

SUMMARY

1. The present paper is a study of several interacting factors which produce varying amounts of black and blue color in pigeons.

2. The basic factor concerned is *B*, producing black pigment.

3. The first group of factors consists of five which belong to the epistatic series. The lowest is B_a, spreading black pigment in the wing bars; the next is S_0, which produces a sprinkling of black in the wing coverts; the third is the checking factor, *C*, producing the well known check pattern; the fourth, *T*, spreads black throughout the wing coverts, leaving a blue tail,—this is the black blue-tail pattern; *S* is the highest member of the series, spreading black pigment throughout the tail, thus producing a full-black.

4. The inheritance of *C* has been amply proven, while that of the other factors is only indicated. All, however, have been shown to lie in an epistatic and not in a multiple-allelomorph series.

5. A factor *U* and its allelomorph determine uniform and light outer vanes in *S* birds, being indistinguishable in the presence of *s*.

6. Five rump conditions studied in black- and blue-tailed birds have been found to be due to a complicated interaction of factors independent of the epistatic series.

7. The density of black pigment is due to a single Mendelizing factor, *D*. This differs from *I* in that it is not sex-linked, and the shades of black resulting from it are not as light as the darkest duns.

8. A check and barred pattern due to two shades of black or dun and not to black and blue or dun and silver have been found to occur when *d* is present. The inheritance of these patterns has not been worked out.

9. The orderly fashion of behavior of the members of the epistatic series led WHITMAN to believe that checks and bars developed orthogenetically.

10. The orderly behavior of these factors is no criterion that the mutations producing them occurred in the same order.

11. If closely related species are found to differ from each other mainly by heritable color factors, they might on such a basis be included in a single species.

The writer wishes to acknowledge her gratitude to Professor COLE for his encouragement and helpful suggestions given during the progress of the work detailed in the preceding pages; to Dr. OSGOOD of the FIELD COLUMBIAN MUSEUM, for permission to examine the specimens of pigeons in his custody at the Museum; and to Mrs. WALDECK of Milwaukee for the loan of the blue barless Homer used in one of the matings.

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SELECTION THROUGH THE CHOICE OF SEEDS FROM DOMINANT PLANTS OF AN ALLOGAMOUS POPULATION[1]

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INTRODUCTION

Consider the problem of selection involved in choosing seeds from plants showing a certain dominant character, while no effort is made to influence the source of pollen. In the following discussion the fate of a single Mendelian character will be studied under such mating.

The conclusion can be stated thus: *The limiting population resulting from the type of mating in question is all of one class, pure dominants.* It is interesting to contrast this conclusion with the fact, repeatedly pointed out in recent years, that in unrestricted random mating the proportions of pure dominants, heterozygotes and recessives are fixed after a single operation of random mating.

DISCUSSION

Let

(1) . . . . $r_{n-1}AA+s_{n-1}Aa+t_{n-1}aa$

represent the $(n-1)$th generation resulting from the type of mating in question, the coefficients being so chosen that

(2) . . . . $r_{n-1}+s_{n-1}+t_{n-1}=1$

The gametes producing the next generation will be in the following proportions:

$$\text{Males:}\quad \frac{(2r_{n-1}+s_{n-1})}{2}A+\frac{(2t_{n-1}+s_{n-1})a}{2}$$

$$\text{Females:}\quad \frac{(2r_{n-1}+s_{n-1})}{2\,K_{n-1}}A+\frac{s_{n-1}a}{2\,K_{n-1}}$$

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[1] This problem was suggested to the author by Mr. L. E. THATCHER, Assistant Agronomist, OHIO AGRICULTURAL EXPERIMENT STATION, Wooster, Ohio. This STATION, under the direction of the Agronomist C. G. WILLIAMS (present Director of the STATION) has carried out the type of selection in question during a period of fifteen years, in experiments with corn. A report of progress was published in 1915 in Ohio Experiment Station Bulletin 282; "Corn experiments." A complete report is now being prepared.

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where $K_{n-1}=r_{n-1}+s_{n-1}$. For convenience we will use for the above proportions,

$$\begin{array}{ll}\text{Males:} & M_{n-1}\,A+N_{n-1}a\\ \text{Females:} & U_{n-1}\,A+V_{n-1}a\end{array}$$

It is evident that the composition of the generation formed by the union of the above gametes is

$$M_{n-1}\,U_{n-1}\,AA+(M_{n-1}\,V_{n-1}+U_{n-1}\,N_{n-1})Aa+N_{n-1}\,V_{n-1}aa$$

The gametes produced by the individuals of this generation, which will enter the mating in question, are in the proportions indicated as follows:

Males:

$$M_n=\frac{2M_{n-1}\,U_{n-1}+M_{n-1}\,V_{n-1}+U_{n-1}\,N_{n-1}}{2}$$

$$N_n=\frac{2N_{n-1}\,V_{n-1}+M_{n-1}\,V_{n-1}+U_{n-1}\,N_{n-1}}{2}$$

Females:

$$U_n=\frac{2M_{n-1}\,U_{n-1}+M_{n-1}\,V_{n-1}+U_{n-1}\,N_{n-1}}{2\,K_n}$$

$$V_n=\frac{M_{n-1}\,V_{n-1}+U_{n-1}\,N_{n-1}}{2\,K_n}$$

in which

(3)........................ $K_n=r_n+s_n$

Replacing

V_{n-1} by $1-U_{n-1}$ and
N_{n-1} by $1-M_{n-1}$

The above relations simplify:

$$M_n=\frac{2M_{n-1}\,U_{n-1}+M_{n-1}\,(1-U_{n-1})+U_{n-1}\,(1-M_{n-1})}{2}$$

$$=\frac{M_{n-1}+U_{n-1}}{2}$$

$$U_n=\frac{2M_{n-1}\,U_{n-1}+M_{n-1}\,(1-U_{n-1})+U_{n-1}\,(1-M_{n-1})}{2K_n}$$

$$=\frac{M_{n-1}+U_{n-1}}{2\,K_n}$$

$$\begin{aligned}K_n&=r_n+s_n\\&=M_{n-1}\,U_{n-1}+M_{n-1}\,V_{n-1}+U_{n-1}\,N_{n-1}\\&=M_{n-1}\,(U_{n-1}+V_{n-1})+U_{n-1}\,(1-M_{n-1})\\&=M_{n-1}+U_{n-1}-M_{n-1}\,U_{n-1}\end{aligned}$$

We thus have the following equations:

(4)........................ $M_n=\dfrac{M_{n-1}+U_{n-1}}{2}$

(5)........................ $U_n=\dfrac{M_{n-1}+U_{n-1}}{2\,K_n}$

(6)........................ $N_n=1-M_n$

(7)........................ $V_n=1-U_n$

(8)........................ $K_n=M_{n-1}+U_{n-1}-M_{n-1}U_{n-1}$

From equation (4) and (5) we deduce,

(9)........................ $K_n=\dfrac{M_n}{U_n}$

It would be desirable at this point to solve the above equations, obtaining the solutions in terms of initial values and the variable n. This has not been accomplished.

Although the fundamental equations have not been solved explicitly, it can be proved, as shown below, that if this type of mating is continued indefinitely the population approaches more and more nearly pure dominant in character.

From equations (4) and (5) we have,

(10)........................ $L_n=\dfrac{L_{n-1}}{2}\left[1+\dfrac{1}{K_n}\right]$,

where $L_n=M_n+U_n$. Similarly,

$$L_{n-1}=\frac{L_{n-2}}{2}\left[1+\frac{1}{K_{n-1}}\right],$$

and so on for decreasing values of n. Hence,

(11).... $L_n=\left[1+\dfrac{1}{K_n}\right]\left[1+\dfrac{1}{K_{n-1}}\right]\left[1+\dfrac{1}{K_{n-2}}\right]\cdots\cdots\left[1+\dfrac{1}{K_2}\right]\dfrac{1}{2^{n-1}}$

From equation (3), $K_n=r_n+s_n$, it is evident that $K_n<1$ for all values of n since from equation (2),
$r_n+s_n+t_n=1$ and t_n is always positive. Hence,

$$1+\frac{1}{K_n}>2$$

for all values of n. Therefore, referring to equation (10) we see that

$$L_n>L_{n-1}$$

for all values of n. But L_n can never be larger than 2, since

$$L_n=M_n+U_n \text{ and } M_n<1 \text{ and } U_n<1$$

It therefore follows that as n increases indefinitely L_n approaches a limit. Hence K_n approaches a limit and

$$\lim_{n=\infty} K_n = 1$$

for otherwise L_n would increase without limit (see equation (11)).
But $K_n = r_n + s_n$ and $r_n + s_n + t_n = 1$.

Hence

$$\lim_{n=\infty} t_n = 0$$

Going back to the method of mating, we note that

$$t_n = V_{n-1} N_{n-1}$$

Hence

(12)........................ $\lim_{n=\infty} V_{n-1} N_{n-1} = 0$

From equations (4), (5), (6) and (7) it is easy to deduce,

(13)........................ $V_n = \dfrac{N_n - N_{n-1} V_{n-1}}{1 - N_{n-1} V_{n-1}}$

From equations (12) and (13) it is evident that V_n and N_n approach limits as n increases indefinitely and that these limits are equal; i.e.,

(14)........................ $\lim_{n=\infty} V_n = \lim_{n=\infty} N_n = 0$

Thus the recessive gamete tends to disappear. Hence both the pure recessive and the heterozygous types tend to disappear as the mating in question is continued indefinitely.

In order to show how rapidly the limiting situation is approached the proportions have been calculated for a particular case. It is assumed that the original cross is between AA and aa, giving a population of the single type Aa. Random mating occurs in this population since no recessives are present to be excluded. The result is the F_2 population indicated by

$$\tfrac{1}{4}AA + \tfrac{1}{2}Aa + \tfrac{1}{4}aa$$

From this point on, selection occurs, since no aa plants are chosen as the source of seeds.

The following table gives the theoretical proportions of the three types of individuals in each generation resulting from the selection in question.

n	r_n	s_n	t_n
1	.0000	1.0000	.0000
2	.2500	.5000	.2500
3	.3333	.5000	.1667
4	.4083	.4667	.1250
5	.4706	.4339	.0956
6	.5226	.4025	.0750
7	.5664	.3736	.0601
8	.6035	.3475	.0491
9	.6352	.3241	.0407
10	.6626	.3032	.0342
11	.6864	.2845	.0292
12	.7072	.2677	.0251
13	.7256	.2526	.0218
14	.7419	.2390	.0191
15	.7565	.2267	.0169
16	.7695	.2155	.0150
17	.7813	.2053	.0134
18	.7920	.1960	.0121
19	.8017	.1874	.0109
20	.8106	.1795	.0099
21	.8187	.1722	.0090
22	.8262	.1655	.0083

Examination of the above table shows that of the twenty-second generation about 83 percent is of the dominant type, less than one percent is of the recessive type and the remainder, over 16 percent, is heterozygous. Furthermore, it will be observed that the proportions are changing very slowly. The indications are that probably twenty more generations would be necessary to raise the proportion of pure dominants to 90 percent of the whole population.

SUMMARY

In selection which consists in the choice of seeds from dominant plants, the composition of the resulting generations will approach more and more nearly pure dominant in character regardless of the composition of the original population if it is not all pure dominant in character.

It is to be noted, however, that the approach to the limiting population decreases in rapidity in succeeding generations.

STERILITY IN WHEAT HYBRIDS. II. CHROMOSOME BEHAVIOR IN PARTIALLY STERILE HYBRIDS

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INTRODUCTION

The cultivated species of wheat can be divided into three definite groups according to their sterility relationships in interspecific crosses (SAX 1921). The Einkorn group contains only one species, *Triticum monococcum;* the Emmer group consists of *T. dicoccum*, *T. durum*, *T. turgidum*, and *T. polonicum;* the Vulgare group consists of *T. Spelta*, *T. vulgare*, and *T. compactum.* Einkorn crossed with members of the Emmer group or with members of the Vulgare group results in F_1 hybrids which are almost if not quite sterile. Species of the Emmer group crossed with species of the Vulgare group result in partially sterile F_1 hybrids. The species within each group are inter-fertile. These sterility relationships are in accord with the recent taxonomic classifications (Tschermak 1914, Percival 1921), with serological relationships as determined by Zade (1914), and with Vavilov's (1914) classification in respect to rust resistance.

In 1917 a study of the chromosomes of the wheat species and their hybrids was undertaken to determine the chromosome relationships in the cultivated species and the chromosome behavior in partially sterile hybrids. A knowledge of the chromosome behavior should be of value both in an analysis of the origin and relationships of the various species and in a genetic analysis of partially sterile hybrids. A cytological and genetic analysis of wide species crosses involving a certain amount of incompatibility, is especially important in view of East's (1921) conclusions in regard to the origin of important domesticated plants and animals.

PREVIOUS CYTOLOGICAL WORK WITH WHEAT

A comparatively large number of investigators have made cytological studies of the chromosome number in wheat. Overton (1893), Körnicke (1896), Dudley (1908), Nakao (1911), Bally (1912, 1919) and Percival (1921) all report 8 as the haploid chromosome number in *T. vulgare*. Spillman (1912) reports about 6 chromosomes in rye and about 40 in wheat. In all of these cases the papers are very poorly illustrated or are not illustrated at all. Percival and Spillman present no drawings or figures to support their conclusions. In the other papers the drawings are not convincing, and if they faithfully represent the preparations the material was undoubtedly poorly fixed. The chromosomes are pictured as more or less shapeless masses and lack detailed shape and structure. Such an appearance is often due to poor fixation.

In 1917 the writer (Sax 1918) found about 28 chromosomes in the first division of the fertilized egg cell in *T. durum*. At about the same time Sakamura (1918) reported the following chromosome counts in wheat: *T. monococcum*, 14 (diploid); *T. durum*, *T. polonicum*, *T. turgidum* and *T. dicoccum*, 28; and *T. Spelta*, *T. vulgare* and *T. compactum*, 42. Sakamura's counts were made almost exclusively from preparations of root tips. The paper contains no illustrations of any kind.

The behavior of the chromosomes in partially sterile wheat hybrids has been described by Kihara (1919). The hybrids used were *T. durum* × *T. vulgare*, *T. turgidum* × *T. compactum*, and *T. polonicum* × *T. Spelta*. The chromosome numbers of the parents were not determined, but were based on the work of Sakamura (1918). The F_1 hybrids were found to have 35 diploid chromosomes, the sum of the gametic number of the parents. The behavior of the F_1 chromosomes in the reduction divisions is described as follows. At the time of the heterotypic division 14 bivalent and 7 univalent chromosomes are formed. The double chromosomes become oriented on the equatorial plate, but some of the single chromosomes may lie outside of the equatorial plate. As the double chromosomes divide normally and pass back to the poles, the single chromosomes become oriented on the plate, divide equationally and join the 14 chromosomes already at either pole. In the homotypic division 14 chromosomes pass to the poles leaving about 7 (often 5 or 6) chromosomes on the plate. These 7 chromosomes do not divide equationally at this time but pass apparently at random to either pole without dividing. Tetrads are usually formed normally but in some cases chromatic bodies are found in the cytoplasm of the one-nucleate microspores. Most of KIHARA's preparations were obtained from anthers, but somatic tissue was also used for determining the chromosome number in the F_1 plants.

KIHARA (1921) has also investigated the chromosome number in the F_2, F_3 and F_4 of the above partially sterile hybrids. In 8 F_2 plants the somatic chromosome number was found to vary from 31 to 42. In 16 F_3 plants the chromosome number varied from 28 to 41, and in 2 F_4 plants the chromosome number was 39 and 42. The number of univalent chromosomes was found to vary from 0 to 5. Some correlation was found in the F_3 between chromosome number and fertility,—the plants with a chromosome number approaching 42 were more fertile than plants with 38 or 39 chromosomes. The paper is illustrated with one plate of microphotographs which, although hardly adequate to support the author's conclusions, do indicate that he had excellent preparations.

It is evident that there is considerable dissension in regard to the chromosome number in wheat. Six investigators report 8 as the haploid chromosome number, one investigator reports about 40, two investigators report 14 for *T. durum*, and one investigator finds 7, 14 and 21 according to the species used. The last investigator (SAKAMURA) does not present any illustrations to support his counts. Since the value of a cytological paper is largely dependent on the character of the illustrations used, it is not surprising that SAKAMURA's results have been questioned (PERCIVAL 1921). In view of the different chromosome numbers reported by various investigators and the paucity of illustrations presented, a thorough cytological investigation of the chromosomes of wheat, with adequate illustrations, is justified.

MATERIAL AND METHODS

The following species of wheat were used in the present investigation:

Species	*Variety*
Triticum monococcum L.	*Hornemanni* Körn.
Cultural species	
T. durum Desf.	*hordeiforme* Körn. (Kubanka)
(*T. aestivum* L. subsp. *durum* (Desf.) Thell.)	
T. polonicum L.	*villosum* Körn. (Polish)
T. turgidum L.	*pseudo-cervinum* Körn. (Alaska)
(*T. aestivum* L. subsp. *vulgare* (Vill.) Thell. var. *turgidum* (L.) Druce)	
T. vulgare Vill.	*lutescens* Körn. (Marquis) *erythrospermum* Körn. (Preston)
(*T. sativum* Lam. *T. aestivum* L. subsp. *vulgare* (Vill.) Thell.)	*albidum* Körn. (Amby) (Bluestem)
T. compactum Host.	*Humboldtii* Körn. (Wash, Hybrid 143.)
(T. *aestivum* L. subsp. *vulgare* (Vill.) Thell. var. *compactum* (Host.) Sacc.)	
T. *Spelta* L. (*T. aestivum* L. subsp. *vulgare* (Vill.) Thell. var. *Spelta* (L.) Sacc.)	*album* Körn.

The chromosome behavior in the following F_1 hybrids was investigated.

(1) *T. monococcum* ♀ × *T. turgidum* ♂
(2) *T. compactum* ♀ × *T. durum* ♂
(3) *T. vulgare* (Amby) ♀ × *T. durum* ♂
(4) *T. durum* ♀ × *T. vulgare* (Amby) ♂
(5) *T. durum* ♀ × *T. vulgare* (Bluestem) ♂
(6) *T. vulgare* (Bluestem) ♀ × *T. durum* ♂
(7) *T. vulgare* (Bluestem) ♀ × *T. turgidum* ♂

For convenience the species of wheat may be divided into the three sterility groups, the Einkorn group, the Emmer (E) group, and the Vulgare (V) group. In measuring pollen grains several additional varieties of *T. vulgare* were used as well as *T. aegilops* Beauv.

The cytological work was started at BUSSEY INSTITUTION in 1917 but was interrupted until 1919 when it was resumed in Illinois. Since 1920 the work has been carried on at the MAINE AGRICULTURAL EXPERIMENT STATION and at the BUSSEY INSTITUTION.

Considerable difficulty was experienced at first in obtaining well fixed material. Often the chromosomes were found clumped together in such a way that accurate counts could not be made. SAKAMURA was unable to obtain well fixed anthers and used root tips for his preparations. Apparently none of the earlier investigators were able to get good fixation of wheat chromosomes at the time of the reduction divisions, if we may judge from the appearance of the illustrations. Poor fixation results in a grouping of the chromosomes into a varying number of shapeless masses of chromatin. I have frequently found approximately 8 such masses in poorly fixed material. In well fixed pollen mother cells the chromosomes are clearly defined, there is little shrinkage, and the cytoplasm shows great detail of structure.

The usual fixatives,—chrom-acetic acid, and Flemming's solution, gave good results in some cases, but the best results were obtained with a modified Bouin's solution developed by ALLEN (1916). This fixative has been used successfully in McCLUNG's laboratory when chromosome counts were desired. The modified Bouin's solution apparently prevents the clumping of the chromosomes and thus facilitates a detailed examination of the individual chromosomes.

In some cases the entire wheat head was fixed when it was about an inch long, but much better results were obtained when the anthers were dissected out. The best fixation was found where the fixative had to penetrate only a thin layer of tissue.

Sections were cut 10–12μ thick and were stained with Haidenhain's iron-haematoxylin. The drawings were done in ink and in all cases were made from single sections. I am greatly indebted to my wife for much assistance with the drawings, to Professor I. W. BAILEY for doing most of the work in preparing the photographs, and to Doctor EAST for valuable suggestions.

THE CHROMOSOMES IN THE CULTIVATED SPECIES OF WHEAT

The chromosomes in the cultivated species of wheat will be considered in order of their sterility and taxonomic relationships.

In Einkorn, *T. monococcum*, there are clearly 7 pairs of chromosomes in the pollen mother cells. At the time of diakinesis the paired chromosomes can be seen twisted about one another (figure 1). Often delicate fibers can be seen between the two members of a bivalent chromosome and between pairs of chromosomes. The bivalent chromosomes shorten, the nuclear membrane disappears, and the chromosomes become oriented on the equatorial plate. A polar view of the chromosomes at this stage is shown in figure 2. In the heterotypic division the spindle fibers have a subterminal attachment to the chromosomes. The division proceeds regularly and there are no lagging chromosomes (figures 3, 4 and 38, plates 1 and 3). Occasionally a bivalent chromosome is drawn out into a long thread during division, as if the two members of the pair were firmly united at one end and were separated only by considerable tension. As the chromosomes pass back to the poles they become distinctly two-parted (figures 4 and 5). At this stage, the late anaphase, the chromosomes can be most readily counted. The numbers of chromosomes are clearly shown in the polar views of the late anaphase by figures 5 and 37. In the telophase the chromosomes lose their identity as individuals and then pass into the resting stage.

The chromosomes of the second or homoeotypic division are long, much like somatic chromosomes, and are difficult to count. The second division is normal and there are no lagging chromosomes (figure 6). This division takes place at right angles to the heterotypic division and in the same plane so that the resulting microspores of each tetrad are in one plane.

In the species of the Emmer group,—*T. durum*, *T. turgidum*, *T. polonicum* and *T. dicoccum*,—there are 14 gametic chromosomes. In *T. durum* and *T. polonicum* the number has been determined in the pollen mother cells, but in other species of this group the number was determined only approximately from somatic counts.

The 14 pairs of chromosomes in the diakinesis of *T. durum* are shown in figure 7. In most cases the double nature of the bivalents is clearly evident. Often some pairs appear to be longer than others but such differences may be due partly to different stages of contraction. The chromosomes on the equatorial plate are shown in figures 8 (*T. polonicum*) and 9 (*T. durum*) and the anaphase is shown in figure 39. When well fixed the chromosomes can be easily counted at these stages. A side view of the metaphase of the heterotypic division is shown in figure 10. The division is regular and there are no lagging chromosomes at any time (figure 11). The two-parted chromosomes on reaching the poles pass into the resting condition and a cell plate is formed (figure 12). The second division is normal and the four cells of the tetrad are formed in one plane (figure 13).

There are 21 haploid chromosomes in the species of the Vulgare group. The chromosomes in the diakinesis are often grouped around the nucleolus, and owing to the relatively large number it is not easy to count them at this stage. Accurate counts can be obtained from the metaphase of the heterotypic division. A polar view of the metaphase of *T. Spelta* is shown in figure 14 and a similar view of the chromosomes of *T. vulgare* is shown in figures 15 and 40. The subterminal attachment of the spindle fibers together with the anastomosis of certain parts of the bivalent chromosomes results in unusual distortion of the chromosomes at the time of division (figure 16). The free ends of the chromosomes are usually short and thick, while the portions between the fiber attachment and the united ends are often drawn out into thin threads. There are no lagging chromosomes at any time either in the anaphase or telophase (figures 17, 19 and 44). The chromosomes in the late anaphase can be easily counted (figure 18). The chromosomes in the second division are very long and can be counted only with difficulty. The approximate number may be counted in figure 20, which is the metaphase of the second division in *T. vulgare*. The division is normal and the tetrads are formed as in the other groups of species.

There is apparently little difference in the size of the individual chromosomes within or between species. Often several long bivalents can be seen in diakinesis in the Emmer and Vulgare groups, but the number of these varies even in the same individual plant. In the side views of the metaphase certain chromosomes are longer than others, but here again the number of very long chromosomes varies and it is probable that the longer chromosomes have no greater volume than some of the shorter ones. In the figures it appears that the individual chromosomes of Einkorn are larger than the chromosomes of the other species, but such an appearance may be entirely due to differences in fixation and staining. The preparations of Einkorn were stained much more deeply than preparations of the other species.

In general there is some correlation between chromosome number and cell size, as indicated by the size of the pollen mother cells in the different species. Often, however, the cells are cut in such a way that a cross section does not indicate their relative volume. Differences in stages of development as well as differences in fixation may also cause differences in apparent size of cells even in the same species.

The chromosomes of rye have never been pictured, so several figures of rye are presented in figures 41A and 41B. The twisted pairs of chromosomes in diakinesis is especially well shown in figure 41A. In several cases all 7 chromosomes can be counted even at the stage of diakinesis.

THE CHROMOSOMES IN STERILE AND PARTIALLY STERILE SPECIES HYBRIDS

T. monococcum × *T. turgidum*

In the cross of Einkorn × Alaska the F_1 plant has 21 somatic chromosomes, 7 contributed by the Einkorn parent and 14 contributed by the Alaska parent. At the time of the first reduction division about seven pairs of chromosomes are formed, leaving approximately 7 single chromosomes. Presumably the 7 Einkorn chromosomes pair with 7 of the Alaska chromosomes, leaving 7 single or univalent chromosomes contributed by the Alaska parent. The bivalent chromosomes orient themselves on the equatorial plate, but the single chromosomes are usually found at or near the poles of the spindle figure,—seldom on the equatorial plate. In figure 22 there are 6 pairs of chromosomes shown on the heterotypic plate and one pair which has divided. Four single chromosomes lie at one pole and three at the other, a total of 7 single chromosomes. The number of single chromosomes at the poles varies somewhat, but the total number is approximately 7 in most cases (table 1).

TABLE 1

Assortment of single chromosomes in the heterotypic division of the F_1 Einkorn × Alaska.

Number of single chromosomes at either pole	4 3	4 4	5 3	4 2	5 2	6 1	5 4	3 2	6 3	5 6
Total singles	7	8	8	6	7	7	9	5	9	11
Actual frequency	8	4	4	3	3	3	2	1	1	1
Theoretical frequency	17				10	3				

The number of single chromosomes may vary due to segmentation, or they may lie on the plate with the double chromosomes in some cases. It is also possible that less than 7 bivalent chromosomes are formed, in which case the total number of singles would be 9 or 11. In general, however, the chromosome number is approximately 7 and the distribution of about equal numbers of single chromosomes to either pole occurs with greatest frequency as expected.

The single chromosomes remain at the poles while the bivalent chromosomes divide and join the single chromosomes. The total number of chromosomes at either pole is then usually about 10 or 11 (fig. 23). As the members of the bivalent chromosomes pass to the pole they split longitudinally as usual (figure 24). All chromosomes both single and double are grouped together in the telophase (figure 25).

In the homoeotypic division approximately ten chromosomes are found at the metaphase (figure 27). These chromosomes are long and curved. They consist of the 7 divided bivalents plus 0 to 7, but usually 2 to 5, univalent chromosomes. Since the univalents did not divide during the heterotypic division they would be expected to divide normally in the homoeotypic division. The actual behavior of the chromosomes during the second division is difficult to determine owing to the length of the chromosomes and the paucity of critical stages. The division appears to be normal, however, and seldom were any lagging chromosomes found (figure 26). The tetrads are formed as in the case of the parental species and in comparatively few cases are chromatin granules found outside of the nuclei. The tetrads separate into one-nucleate pollen grains which appear to be normal. Very few of the one-nucleate pollen grains develop normally, however, and only about 2 to 3 percent of the mature pollen grains appear normal. Probably none of them is functional.

In a few cases the univalents were found on the heterotypic plate after the bivalents had divided, indicating that the behavior of the univalents in the reduction division may vary in different individuals.

Emmer group × *Vulgare group*

In crosses between members of the Emmer group and members of the Vulgare group the sum of the gametic numbers of chromosomes is 35. Such F_1 plants will have 14 chromosomes contributed by one parent and 21 chromosomes contributed by the other parent. In the pairing of the chromosomes for reduction the 14 chromosomes of the Emmer group presumably pair with 14 chromosomes of the Vulgare group, leaving 7 univalent Vulgare chromosomes. At any rate in diakinesis there are about 14 bivalents and 7 univalents. The bivalent and univalent chromosomes can easily be distinguished in the metaphase of the heterotypic division. A polar view of the metaphase of the F_1 of Amby (V) × Kubanka (E) is shown in figures 28 and 42. The 7 univalent chromosomes are long, slender and V-shaped, while the bivalent chromosomes are like the bivalents in the normal heterotypic division in the parent species. A similar stage is shown in the F_1 of Bluestem (V) × Alaska (E) in figure 29. The univalents are usually arranged on one side of or around the group of bivalents.

Some of the single chromosomes often lie outside of the equatorial plate and appear to be distributed more or less at random in the division figure (figure 30). Usually only two or three single chromosomes can be seen outside of the equatorial plate in a side view, but in a polar view all of the univalents may lie outside of the group of bivalents. As the bivalents divide and pass to the poles the univalent chromosomes become oriented on the equatorial plate (figures 31-34, 43 and 45). The univalents may vary in number due possibly to the failure of all 14 chromosomes to pair, or to the premature or accidental passage of single chromosomes to the poles (figure 30).

TABLE 2

Number of lagging single chromosomes in the heterotypic division of F_1 *of Emmer group × Vulgare group.*

Number of lagging chromosomes	4	5	6	7	8	9
Frequency	1	3	7	22	2	1

Table 2 was prepared from well fixed division figures before all of the univalents had separated and where the figure was apparently intact. In the majority of cases 7 lagging univalents were found (figures 32 and 34). In one case 9 single chromosomes appeared to be present (figure 31), but at least one univalent may be segmented into two parts. The univalents are frequently somewhat constricted in the centers where the spindle fibers are attached. The grouping of the bivalents on one side of the spindle is often found (figure 33). The 14 chromosomes at the poles, resulting from division of the bivalents, can frequently be counted with little difficulty, but it is difficult to picture them because they are so compactly grouped.

The segmented univalent chromosomes divide and pass to the poles (figure 35). In most, if not in all cases, there are no chromosomes left free in the cytoplasm, but all are grouped together in the telophase (figures 36 and 46). The daughter nuclei thus formed pass into the resting stage.

In the second division some univalent chromosomes are found outside of the equatorial plate in the metaphase (figure 47). In the late anaphase or telophase univalent chromosomes are found lagging after most of the chromosomes have reached the poles (figures 37 and 48). The number of lagging chromosomes varies, with the mode at 5. Perhaps some of the chromosomes at the poles in the metaphase (figure 47) remain there and fail to take their place on the plate, or single chromosomes may accidentally pass to the poles with the original members of the bivalents. The following table shows the distribution of the single lagging chromosomes in the second division.

TABLE 3

Number of lagging chromosomes in the homoeotypic division. Vulgare group × Emmer group.

Number of lagging chromosomes	2	3	4	5	6	7
Frequency	1	3	5	10	5	2

The lagging chromosomes do not divide equationally but pass apparently at random to either pole. Thus the four microspores each contain 14 chromosomes presumably contributed by both parents according to chance and from 0 to 7 additional chromosomes of the Vulgare parent, the latter number also depending on chance distribution. The fact that the univalent chromosomes divide but once, equationally of course, while the bivalents divide twice in the course of gametogenesis, is in accord with the usual conception of the nature of the reduction divisions.

In some cases the lagging chromosomes fail to go back to the poles and several chromosomes may lie outside of the reconstructed nucleus, usually in the vicinity of the newly formed cell wall. As a rule, however, microspores are formed which have no chromatic material in the cytoplasm outside of the nucleus. These one-nucleate pollen grains appear to be entirely normal so far as the morphological structure is concerned. In many cases, however, they are unable to develop into normal pollen grains. About 20 percent of the pollen of the F_1 plants resulting from Emmer group × Vulgare group crosses is obviously imperfect as indicated by smaller size and meager contents. Undoubtedly a larger percentage is not functional because of physiological or other imperfections in pollen grains that appear morphologically perfect.

THE POLLEN GRAINS IN PARENTS AND F_1 HYBRIDS

There is a striking correlation between chromosome number and the size of the pollen grains in the wheat species studied. Such a relation would be expected if BOVERI'S conclusions concerning chromosome number and cell size have a general application. The pollen grains are especially good for testing such relationships because they consist of only several cells, their volumes are easily determined, and they can be meas-

TABLE 4

Size and variability of pollen grains of wheat varieties and species and of some F_1 hybrids. 1921 data.

VARIETY OR F_1 HYBRID		DIAMETER IN MICRONS (CLASS CENTERS)																					N	MEAN	σ	C. V.	VOLUME CU. μ
		37.5	39.0	40.5	42.0	43.5	45.0	46.5	48.0	49.5	51.0	52.5	54.0	55.0	57.0	58.5	60.0	61.5	63.0	64.5	66.0	67.5					
Aegilops ovata	W		1	8	15	15	16	5	2	1													63	43.55±.18	2 12±.13	4.87±.29	53,000
	L								1	3	5	19	20	9	5	1							63	53 55±.15	1.80±.11	3.35±.20	
Einkorn.............			3	12	23	33	44	13	6	4													138	44.02± 13	2.19±.09	4.98±.20	45,000
Kubanka	E						1	3	13	19	20	22	10	4	3	1							96	51.22±.18	2.62±.13	5.12±.24	70,000
Alaska	E						2	4	8	13	14	23	22	7	1	1							95	51.76±.19	2 68±.13	5.18±.25	73,000
Polish	E					1	5	6	19	17	27	31	5	1	1								113	50.34±.16	2.47±.11	4.90±.22	67,000
Emmer	E						2	2	8	19	29	26	11	9	4	2							112	51.70±.17	2.62±.12	5.07±.23	72,000
Spelt	V								2	2	4	13	15	17	11	12	8	3	1				88	55.59±.23	3.15±.16	5 67±.28	90,000
Marquis	V									2	5	18	20	27	18	5	4						99	54.91±.15	2.27± 11	4.14±.20	87,000
Amby	V									1	5	14	16	26	15	8	4	2					91	55.30±.17	2 47±.12	4.46±.22	89,000
Preston	V									2	4	21	23	25	20	5	5						105	54.92±.15	2 29±.11	4.17±.19	87,000
Royalton White	V									1	5	15	24	33	14	7	2						101	54 92±.14	2.06±.10	3.75±.14	87,000
Kota V × Royalton	V							1	1	3	7	12	17	21	18	8	4		1				93	54.95±.20	2.85±.14	5.19±.25	87,000
Spelt V × Marquis	V			1	1	1	2	1	6	4	8	9	12	18	17	13	16	5	4	1			119	55.37±.29	4.67±.20	8.44±.37	89,000
Polish E × Emmer	E				1	3	2	7	8	10	13	18	15	12	9	1	1		1				101	51.94±.26	3.85±.19	7.40±.35	73,000
Emmer E × Polish	E			1	1	2	2	4	9	9	9	24	12	10	10	5	3	2	2				105	52.73±.29	4.37±.20	8.28±.39	77,000
Kubanka E×Marquis	V	1	2	3	4	8	8	4	4	3	7	15	10	6	7	11	12	6	4	4	1		120	53.04±.43	6.39±.30	13.07±.58	78,000

ured at the same stage of development. The size and variability of the pollen grains of most of the wheat species and of F_1 hybrids between varieties, compatible species, and members of different sterility groups are shown in table 4.

In *T. aegilops ovata* the pollen grains were not spherical and two dimensions were taken to determine the volume. In all other cases the pollen grains were approximately spherical, so only the diameter was measured. In all cases only the apparently good pollen grains were used. Only in the partially sterile hybrid Kubanka × Marquis, was the percentage of obviously poor pollen grains more than 1 to 2 percent. The pollen grains were mounted in lactic acid and were measured with an ocular micrometer.

In Einkorn with 7 chromosomes the mean volume of the pollen grains, measured in thousands of cubic microns, is 45. In the species of the Emmer group with 14 chromosomes the mean volume of the pollen grains is about 70. In the species of the Vulgare group the mean volume of the pollen grains is about 88. The F_1 hybrids resulting from crossing members of the Vulgare group, have pollen grains of about the same size as the parents, and a similar condition obtains for F_1 hybrids within the Emmer group.

Not only is there a consistent and significant relation between pollen-grain size and chromosome number in the various species groups, but there is also a consistent relation between heterozygosis and pollen-grain variability. In the homozygous parents the coefficient of variability for pollen-grain diameter ranges from $3.75 \pm .14$ to $5.67 \pm .28$. In the cross, Kota × Royalton White, both parents belong to the same species, *T. vulgare*, and the pollen-grain variability is little if any greater than for the parents. In the cross, Spelt × Marquis, the parents belong to different species, but to the same sterility group, and the pollen of the F_1 is significantly more variable than for the parents or the preceding varietal cross. The reciprocal crosses between Polish and Emmer also involve different species, but only one sterility group, and the pollen-grain variability in the F_1 is significantly greater than in the parents. The F_1 resulting from Kubanka × Marquis has pollen grains almost three times as variable as the parent species. In this case the parents belong to different sterility groups and the F_1 is partially sterile. The striking increase in pollen-grain variability is not due to obviously aborted pollen grains because only the apparently good pollen grains were measured It will be noted that the distribution is not especially skew or bimodal as would be the case if the increased variability were due only to poorly developed or aborted pollen.

Apparently environmental conditions may cause differences in pollen-grain size. The size of the pollen grains in 1920, measured in thousands of cubic microns, was 72 for Einkorn, 94 for the Emmer group and 114 for the Vulgare group. The relative sizes of the pollen grains of the three sterility groups was about the same in 1920 and 1921.

Pollen-grain size and chromosome number are also correlated in a similar manner in the species of oats.

DISCUSSION

Polyploidy in plant species

The occurrence of the gametic chromosomes in multiples of 7 in the species of wheat suggests that the Emmer and the Vulgare groups are tetraploid and hexaploid forms, respectively, of an original type with 7 chromosomes. The origin of new species by tetraploidy in other genera would tend to support such a view. *Oenothera gigas*, a mutant from *Oe. Lamarckiana*, is the best known case of tetraploidy in plants. The chromosome number of the mutant was found to be 28 as compared with 14 for the parental species (LUTZ 1907). Miss DIGBY (1912) found a tetraploid form of *Primula kewensis* with 36 somatic chromosomes. Tetraploidy has been found recently in Datura (BLAKESLEE, BELLING AND FARNHAM 1920). In all of the above cases tetraploidy has occurred while the species were under observation. The hereditary behavior of the tetraploid plants shows a doubling of genetic factors and thus supports the cytological observations.

Chromosome duplication has also been found in other species which have not been under such close observation and which have not been tested by breeding experiments. In Hieracium there are species with 18, 27, 36 and 42 somatic chromosomes (ROSENBERG 1917). In Crepis species are found with 6, 8, 10, 16, 18, 24 and 42 somatic chromosomes. ROSENBERG (1920) believes that the species with the higher chromosome numbers are caused by the duplication of one or more pairs of the chromosomes in the primary species, *C. virens*, with 3 pairs of chromosomes. In the species with 8 chromosomes the medium-sized pair of chromosomes is reduplicated while in the "*gigas*-mutant," *C. Reuteriana*, each pair is reduplicated. In the genus Rosa species are found with 14, 21, 28, 35 and 42 somatic chromosomes (TÄCKHOLM 1920).

In Chrysanthemum TAHARA (1915) finds in different species haploid numbers of 9, 18, 27, 36 and 45. GATES (1913) lists a number of other cases of tetraploidy in plant and animal species and additional cases have been found in the past few years.

Genetic behavior of polyploid species

In tetraploid plants the doubling of the chromosome number results in a duplication of the genetic factors. Thus tetraploidy can be detected not only by chromosome counts but also by the genetic behavior of hybrids of tetraploid races. If the reduplicated chromosomes of a tetraploid or hexaploid species, or to use BLAKESLEE'S (1920) terminology, the members of the tetrasomic and hexasomic sets, assort in pairs, the usual 15 : 1 or 63 : 1 ratios will result in F_2. If, however, the chromosomes of a tetrasomic or hexasomic set assort at random in an F_1 hybrid a 35 : 1 or 399 : 1 ratio will be found in the F_2. Furthermore, in such tetraploid and hexaploid plants the number of recessive factors may often exceed the number of dominant factors in heterozygous individuals in F_2 hybrids. There is evidence that in some cases either allelomorph of a pair of characters may be dominant if an excess of factors of one character are present. In the case of starchy and corneous endosperm in maize, HAYES and EAST (1915) have found that the character borne by the mother plant was always dominant, due presumably to the dominance of the two factors contributed by the mother, as a result of polar fusion, over the single factor contributed by the male parent. In Drosophila it has been found that the two recessive genes for vermilion and sable dominate the normal allelomorphs. When the mutant and normal factors are in equal numbers the normal is dominant (MORGAN 1919). Sex in Drosophila is determined by the ratio of X chromosomes to autosomes. BRIDGES (1921) has found that the ratio of 2 X : 2 sets of autosomes produces a female while 1 X : 2 sets of autosomes produces a male and the ratio 2 X : 3 sets of autosomes produces an intermediate condition, the intersex. There is also evidence that characters other than sex are also influenced by chromosome ratios.

It is possible that an excess of recessive factors may be dominant over the normally dominant factors in many cases, but because such cases become evident in diploid species only in endosperm characters, or in case of non-disjunction or other unusual chromosome behavior, they are seldom detected. The dominance of the normally recessive character would never occur in diploid plants except in endosperm characters, but in triploid, tetraploid and hexaploid plants such a relationship of dominant and recessive characters would be found. Although few such cases have been discovered it is a possibility to be considered. We have then two possible causes for unusual genetic ratios in polyploid plants,—random assortment of chromosomes and reversed dominance due to an excess of recessive factors. The gametic constitution and genetic ratios for various combinations of diploid, tetraploid, and hexaploid hybrids are given in table 5.

In case of random assortment of chromosomes in a tetrasomic set the F_2 ratio becomes 35 : 1 instead of 15 : 1 which is found if the chromosomes assorted in pairs. In the hexasomic set random assortment would result in an F_2 ratio of 399 : 1 and in most F_2 plant populations such a segregation would not be detected. It is also obvious that a tetraploid individual can never be homozygous for a single factor in case of random chromosome assortment and in hexaploid individuals neither one nor two factors can exist in the homozygous condition. If, then, a mutation occurs in a single chromosome of a hexasomic set in which the chromosomes assort at random the homozygous condition will be attained only when all 3 chromosomes of the set are either dominant or recessive. In a homozygous hexaploid race one- and two-factor differences will not be found with random assortment of the members of the hexasomic sets.

An examination of the hereditary behavior of tetraploid species indicates that in most cases studied the chromosomes assort at random in the tetrasomic sets. MULLER (1914) in analyzing GREGORY'S data on tetraploid Primulas concluded that where more than 2 factors are present, which are normally allelomorphic to each other, the pairing of these allelomorphs usually takes place at random. He interpreted the ratio of 4.5 : 1 in a back-cross as a 5 : 1 ratio expected, rather than a 3 : 1 ratio, and showed that the ratio of 119 : 4 in F_2 may be interpreted as a 35 : 1 ratio rather than a 15 : 1 ratio proposed by GREGORY. MULLER'S analysis of GREGORY'S data indicates that in the tetraploid Primula the chromosomes of a tetrasomic pair assort at random. In Datura the duplicated chromosomes of the Poinsettia mutant apparently assort at random according to BLAKESLEE, BELLING AND FARNHAM (1920). Unusual ratios are obtained because the Poinsettia character is not carried by the pollen to any significant extent. In *Oenothera gigas* certain races when selfed produce 1 to 2 percent *nanella* mutants and pedigrees are found approaching a 3 : 1 ratio. BLAKESLEE'S (1921) analysis of DE VRIES'S data indicates that the 1 to 2 percent *nanella* segregates are recessive in a 35 : 1 ratio, and the fact that dominants from a 3 : 1 ratio throw 3 : 1 ratios or 35 : 1 ratios supports BLAKESLEE'S

TABLE 5

Result of crossing diploid, tetraploid, and hexaploid species when (1) the allelomorphic chromosomes unite in homologous pairs, and when (2) they pair at random. The F_2 ratios, $R : r$, are given when (a) an Rrr genotype appears as an R phenotype and when (b) an Rrr genotype appears as an r phenotype.

		CHROMOSOMES UNITE IN HOMOLOGOUS PAIRS			CHROMOSOMES OF POLYSOMIC SET PAIR AT RANDOM		
Parents	F_1	F_1 gametes	(a)	(b)	F_1 gametes	(a)	(b)
$\begin{matrix}R_1R_2\\R_1R_2\end{matrix}\times\begin{matrix}r_1\\r_1\end{matrix}$	R_1R_2 r_1	R_1R_2-r_1-R_2-r_1R_2	15 : 1	13 : 3	R_1R_2-R_1r_1-r_1R_2-r_1-R_1-R_2	35 : 1	31 : 5
$\begin{matrix}R_1\\R_1\end{matrix}\times\begin{matrix}r_1r_2\\r_1r_2\end{matrix}$	R_1 r_1r_2	R_1-r_1r_2-R_1r_2-r_1	3 : 1	3 : 5	r_1r_2-r_1R_1-r_2R_1-R_1-r_1-r_2	3 : 1	13 : 23
$\begin{matrix}R_1R_2\\R_1R_2\end{matrix}\times\begin{matrix}r_1r_2\\r_1r_2\end{matrix}$	R_1R_2 r_1r_2	R_1R_2-R_1r_2-r_1R_2-r_1r_2	15 : 1	11 : 5	R_1R_2-R_1r_2-r_1R_2-r_1r_2-R_1r_1-r_1R_2	35 : 1	3 : 1
$\begin{matrix}R_1R_2R_3\\R_1R_2R_3\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	$R_1R_2R_3$ $r_1r_2r_3$	$R_1R_2R_3$-$r_1R_2R_3$-$R_1r_2R_3$ $R_1R_2r_3$-$r_1r_2r_3$-$R_1r_2r_3$ $r_1R_2r_3$-$r_1r_2R_3$	63 : 1	42 : 22	$R_1R_2R_3$-$r_1R_2R_3$-$R_1r_2R_3$-$R_1R_2r_3$ $r_1r_2r_3$-$R_1r_2r_3$-$r_1R_2r_3$-$r_1r_2R_3$ $R_1R_2r_2$-$r_1R_3r_3$-$r_1R_2r_2$-$R_1R_3r_3$ $R_2R_1r_1$-$r_2R_3r_3$-$r_2R_1r_1$-$R_2R_3r_3$ $R_3R_1r_1$-$r_3R_2r_2$-$r_3R_1r_1$-$R_3R_2r_2$	399 : 1	282 : 118
$\begin{matrix}R_1R_2r_3\\R_1R_2r_3\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	$R_1R_2r_3$ $r_1r_2r_3$		15 : 1	22: 42		24 : 1	2 : 3
$\begin{matrix}R_1r_2r_3\\R_1r_2r_3\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	$R_1r_2r_3$ $r_1r_2r_3$		3 : 1	0 : 64		3 : 1	0 : 400
$\begin{matrix}R_1R_2R_3\\R_1R_2R_3\end{matrix}\times\begin{matrix}R_1r_2r_3\\R_1r_2r_3\end{matrix}$	$R_1R_2R_3$ $R_1r_2r_3$		64 : 0	15 : 1		400 : 0	24 : 1
$\begin{matrix}R_1R_2R_3\\R_1R_2R_3\end{matrix}\times\begin{matrix}r_1r_2\\r_1r_2\end{matrix}$	$R_1R_2R_3$ r_1r_2		63 : 1	3 : 1		399 : 1	324 : 76
$\begin{matrix}R_1R_2\\R_1R_2\end{matrix}\times\begin{matrix}r_1r_2r_3\\r_1r_2r_3\end{matrix}$	R_1R_2 $r_1r_2r_3$		15 : 1	26 : 38		3 : 1	1 : 24

conclusions. Blakeslee suggests that all of the Oenothera mutants are caused either by chromosomal duplication or are due to crossovers from balanced lethals as suggested by Muller (1918). In connection with Muller's hypothesis, however, it may be suggested that if we accept the telosynaptic method of reduction described in Oenothera by Davis (1911) and Gates (1915) and at the same time accept the mechanical chiasmatype hypothesis of crossing over, there would be little or no opportunity for crossing over to occur.

In the case of Oenothera, Primula and Datura, the tetraploid forms have originated in experimental cultures and in all cases the chromosomes of a tetrasomic set appear to assort at random. In wheat there is no evidence that random assortment of chromosomes takes place in tetrasomic and hexasomic sets. The results of Nilsson-Ehle (1909), the Howards (1912) and Gaines (1917) all show ratios closely approximating 15 : 1 or 63 : 1 in wheat hybrids involving several independent similar, or possibly identical, factors for the same character. Segregates can be obtained from the F_2 of a trihybrid, which are homozygous for 1 or for 2 factors, an impossible result in case of random chromosome assortment. In the segregation of capsule form in Bursa as described by Shull (1914) the F_2 ratio approximately 24 : 1 might suggest a random assortment of chromosomes in a hexasomic set with 4 dominant factors, but such a parental type would not exist in a homozygous condition and the behavior of the F_2 segregates proves that 2 factors are involved which assort in pairs. The fact that a character may be determined by several factors does not necessarily indicate that these factors are the result of chromosome duplication.

In both wheat and Bursa multiple factors, presumably allelomorphic and in duplicate chromosomes, do not assort at random as they do in case of tetraploid Oenotheras, Primulas and Daturas. The difference in chromosome behavior may be due to the recent origin of tetraploidy in some species as compared with wheat. It is also possible that the increased chromosome number is not due to duplication of the fundamental chromosome number, or if it is, the individual chromosomes may have changed so that they assort in pairs.

Chromosome behavior in certain partially sterile hybrids

Hybrids between diploid and tetraploid, and between tetraploid and hexaploid, wheat species are completely or partially sterile, and irregular chromosome behavior occurs in the reduction divisions. In the F_1 hybrids between Einkorn with 14 chromosomes and a member of the Emmer group with 28 chromosomes there are 7 bivalents and 7 univalents in the heterotypic spindle. The bivalents divide normally in both meiotic divisions while the singles pass at random, without dividing, to either pole in the first division and presumably divide in the second division. In hybrids between members of the Emmer group with 14 chromosomes with members of the Vulgare group with 21 chromosomes the 14 pairs of chromosomes divide as usual in meiosis while the 7 univalents lag behind but ultimately divide equationally in the first division and pass at random, without dividing, to either pole in the homoeotypic division. A comparison of chromosome behavior and sterility in similar hybrids in other genera is of interest.

In Drosera (ROSENBERG 1909) the chromosomes of the F_1 of a cross between a species with 10 haploid chromosomes and a species with 20 chromosomes, behave like the chromosomes in the Einkorn × Alaska cross in wheat. In the reduction division 10 bivalents and 10 univalents are found. The single chromosomes lag and apparently go at random to either pole without dividing. The second reduction division is not described.

In the F_1 of *Oenothera lata* × *Oe. gigas* GATES (1909) found the chromosome number to be 20 or 21, the sum of the gametic numbers of the parents. In the reduction division 10 or 11 paired chromosomes were found on the heterotypic spindle. The division is somewhat irregular in some cases, but DAVIS (1911) has found a rather loose association of the chromosomes in the parental types. GEERTS (1911), however, found 7 bivalents and 7 univalents in the reduction division of the F_1 of *Oe. Lamarckiana* × *Oe. gigas*. The single chromosomes pass to either pole without dividing, usually 3 to one pole and 4 to the other. In the second division the univalents also divide irregularly.

The number of somatic chromosomes in species of Hieracium ranges from 18 to 42. In *H. excellens* (ROSENBERG 1917) with 42 chromosomes, 18 pairs of chromosomes are found on the heterotypic spindle in addition to 6 univalents. In the F_1 of *H. auricula* (18 chromosomes) × *H. aurantiacum* (36 somatic chromosomes), 9 bivalents were found with 8 or 9 univalents in the reduction division. The univalents divide irregularly. Similar chromosome behavior was found in other hybrids but often the number of univalents was found to vary even in the same cross.

TÄCKHOLM (1920) has found only paired chromosomes in the series 7, 14 and 21, in some Rosa species. Other species, especially those of the Canina section, have both bivalents and univalents at the time of reduction, usually 7 bivalents with 14, 21 or 28 univalents. The species which have both bivalents and univalents in pollen reduction are thought to be hybrids which have been perpetuated for long periods of time by apomictical reproduction.

The behavior of the chromosomes in reduction divisions of species of Rosa has also been described in considerable detail by BLACKBURN and HARRISON (1921). In *R. Sabini* (*R. pimpinellifolia* × *R. sylvestris*) with 42 chromosomes, there are 14 bivalents and 14 univalents on the heterotypic spindle. The univalents lag behind in division but ultimately divide and pass to the poles. In the second reduction division the original bivalents pass rapidly to the poles and the daughter nuclei are reconstructed before the original singles reach the poles. Thus the major nuclei contain about 14 chromosomes. The univalent chromosomes form micronuclei and often a total of 8 nuclei are found which may be termed an "octad."

In one case in the above hybrid all of the chromosomes at the time of the homotypic division were on a single spindle. If development had been allowed to proceed the gametes would be able to produce a new plant octoploid in chromosome number.

In other Rosa hybrids BLACKBURN and HARRISON find a type of reduction similar to that found in *R. Sabini*, but involving 7 bivalents and 21 univalents or 14 bivalents and 21 univalents, etc. All roses showing such partial reduction are facultatively apomictical, due, according to the authors, to the stimulus of heterozygosis.

In Canna and Datura (BELLING 1921) the chromosome behavior in triploid individuals is quite different from that found in similar cases in Triticum, Rosa, Drosera, and Hieracium. In one clone of Canna the 27 chromosomes unite into 9 triads at reduction. The chromosomes of each triad pass at random, two to one pole and one to the other.

Among species hybrids in animals the chromosome behavior in F_1 reduction may vary greatly. FEDERLEY (1915) crossed two species of butterflies, each with 28 chromosomes. In the F_1 the chromosome number varied from 28 to 33 in spermatogensis, due to the varying number of single and double chromosomes on the plate. FEDERLEY believes that there is a negative correlation between the number of paired chromosomes and sterility in such cases. In another species cross FEDERLEY (1916) found little or no failure of the chromosomes to pair in reduction. In the F_1 of *Pygaera curtula* L. (29 chromosomes) with *P. anachoreta* F. (30 chromosomes) FEDERLEY (1913) found about 59 chromosomes in the reduction division, indicating that little or no pairing of chromosomes occurred. In a back-cross of the F_1 with *P. anachoreta* the chromosomes of the same species paired leaving 29 single *curtula* chromosomes. The univalents divided equationally in both divisions, but the second division was often abnormal.

In general, triploid hybrids derived from species with different chromosome numbers have a similar type of chromosome behavior in the reduction divisions. Usually the number of bivalents is equal to the gametic number of chromosomes found in the parent with the smaller number, and the number of univalents is the difference between the chromosome numbers of the parental species. The bivalents divide normally while the univalents usually divide but once, equationally of course, and pass at random to either pole without dividing in one of the reduction divisions. Whether the single chromosomes divide in the first or second reduction division should make but little difference so far as the genetic results are concerned, providing that they divide but once. In case some or most of the univalents are not included in the functional pollen grains, as found in Rosa, the genetic behavior will be disturbed.

Chromosome behavior and sterility

The occurrence of a triploid chromosome number or the reduplication of a single chromosome is associated with more or less sterility. The relation of chromosome number and behavior to sterility is shown for Oenothera, Datura, and Triticum in table 6.

The percentage of apparently poor pollen grains as an indication of sterility is, of course, only an approximate measure, but for general purposes it is considered satisfactory. In Oenothera the species and hybrids having an additional chromosome or those which are triploid are much more sterile than the diploid or tetraploid types. Here, of course, the sterility may be disturbed also by the hybrid nature of most of the Oenotheras or due to balanced lethal factors. In Datura the reduplication of but a single chromosome results in some sterility, still greater sterility is found in the triploid individual, while the tetraploid species is as fertile as the diploid parental type. Tetraploid and hexaploid species of wheat are as fertile as diploid species. In pentaploid hybrids with 14 bivalents and 7 univalents the sterility is much less than in triploid hybrids with 7 bivalents and 7 univalents. The degree of sterility may, however, be greater than indicated by the appearance of the pollen grains. Sterility based on grains set per spikelet shows total sterility for the triploid hybrid and somewhat greater than 50 percent sterility in the pentaploid hybrid as compared with the parents. If we take into account the effect of hybrid vigor and compare the F_1 sterility

TABLE 6

Relation between chromosome number and sterility.

	CHROMOSOME NUMBER	AVERAGE PER-CENT OF POOR POLLEN
Oenothera (GATES 1915)		
Oe. gigas	28	54–72
Oe. Lamarckiana	14	42
Oe. lata rubricalyx × *rubricalyx* segregate	14	22
Oe. lata rubricalyx	14	33
Oe. semilata	15	77
Oe. lata-like	15	76
Oe. rubricalyx × *gigas*	21	81
Oe. gigas × *lata rubricalyx*	22	89
Datura (BLAKESLEE 1921)		
Normal	24	3
"New species"	48	3
Triploid	36	34
Trisomic (12 mutants)	25	8–21
Triticum (SAX 1921)		
Vulgare group	42	1
Emmer group	28	2
Einkorn group	14	1
Vulgare × Emmer F_1	35	12–22
Einkorn × Emmer F_1	21	98

with the grain set in fertile species crosses, the degree of sterility may be considered as about 60 to 70 percent in the pentaploid hybrids (see SAX 1921, table 4). The degree of sterility in pentaploid hybrids varies in different species crosses and the above is only a general statement of the facts. In Rosa there is also a high degree of correlation between chromosome duplication and sterility.

In all of the above cases the reduplication of both sets of parental chromosomes does not cause sterility or abnormal chromosome behavior. The triploid hybrid resulting from a cross of a diploid with a tetraploid species or a triploid individual resulting from other causes is characrized by abnormal chromosome behavior and sterility. In wheat, sterility is greater where the proportion of univalent to bivalent chromosomes is 1 : 1 than where the ratio is 1 : 2. In Oenothera, Datura, and in one case in Rosa sterility is associated with the reduplication of a single chromosome. The uniform relation between chromosome behavior and sterility in these cases indicates that similar factors are involved.

In some species hybrids the F_1 is completely sterile even though the parental chromosomes may be very similar. For instance *Crepis tectorum* is thought to have the same chromosome constitution as *C. virens*, but with one reduplicated pair (ROSENBERG 1919), but BABCOCK and COLLINS (1920) have found that a cross between the two species results in an F_1 individual which cannot even complete its vegetative development. Still more remarkable are the recent results of STURTEVANT (1921) with Drosophila. Seven mutant genes of *D. simulans* have been shown to be allelomorphic to mutant genes of *D. melanogaster*, but crosses of these species with a similar chromosome constitution results in sterile F_1 individuals. In other cases species with apparently similar chromosome constitution cannot even be crossed.

Sterility in wheat hybrids

A brief summary of the behavior of the F_2 generation of partially sterile hybrids will be of value in interpreting the correlation between chromosome behavior and sterility. In 1917, 84 seeds from an F_1 plant of Kubanka × Bluestem were planted in the greenhouse at BUSSEY INSTITUTION. Six seeds did not germinate; 18 germinated and grew but did not pass the rosette stage; 61 grew and formed heads, but 14 of these were completely sterile. Of the 47 plants which set grain only one or possibly 2 were as fertile as the parents. The average number of grains per spikelet for the 61 plants that headed was .82 ± .05 as compared with an average of .56 for 30 F_1 plants and 2.45 for the parents. The correlation between sterility and height of the 61 plants that headed was $-.42 \pm .07$, indicating that the more sterile plants were poorly developed vegetatively.

The greater sterility of certain F_2 segregates as compared with the F_1 is shown also in a cross of *T. compactum* × *T. turgidum* grown by GAINES in 1921. The average grains per spikelet for the 293 F_2 plants that headed, was .63 ± .02 as compared with 1.09 ± .05 grains per spikelet for the F_1. In the F_1 no plants were found in the sterility class .0—.2 grains per spikelet, while in the F_2 123 plants were in the lowest sterility class. The conditions under which the F_2 was grown were more favorable than for the F_1. Furthermore a large proportion,—probably more than half,—of the F_1 seed did not grow or did not produce plants which headed.

The relation of chromosome behavior to sterility in wheat hybrids

Let us now gather the facts that bear on the relation of chromosome behavior to sterility and to the origin and the relationships of the various species of wheat. In general, sterility increases as the proportion of univalent chromosomes in the reduction division increases. In both triploid and pentaploid wheat hybrids the 7 univalents apparently pass at random to either pole in one of the reduction divisions. The degree of sterility may vary in pentaploid hybrids involving different parental species (HAYES, PARKER and KURTZWEIL 1920, SAX 1921). Many F_2 individuals may be less fertile than the F_1, but in the F_2 sterility is also associated to some extent with poor vegetative development. With the above cytological and genetic data it should be possible to suggest a cause of sterility in wheat hybrids.

If we assume that the tetraploid and hexaploid species are due simply to a reduplication of the chromosomes of a primary diploid species with only minor changes in the individual chromosomes, then the sterility must be due to quantitative relations between univalents and bivalents rather than any specific constitution of individual chromosomes. That such may be the case is suggested by the chromosome behavior in other genera where the univalents are known to be reduplicated chromosomes of a diploid species. With 7 univalent chromosomes assorting at random only 1 gamete in 64 would contain all or none of the univalents. If only gametes containing all or none of the univalents can develop and function, sterility would be almost complete. Such a relation of the chromosomes would explain practically all of the sterility found in triploid wheat hybrids, but it would not explain the greater fertility found in pentaploid wheat hybrids. Possibly the greater number of bivalents in pentaploid hybrids would permit normal development of gametes with an excess or deficiency of several univalents necessary for a diploid or triploid chromosome combination. If only gametes with 3 or 4 univalents, i. e., gametes with 17 or 18 chromosomes, failed to function, then somewhat more than half of the gametes would be sterile, which in general would agree with the degree of sterility in the F_1. Gametes with larger chromosome numbers might be expected to develop with a greater deviation from the normal gametic number than gametes with a small number of bivalents. In the F_2 individuals the number of univalents would never exceed the ratio found in the F_1 if homologous chromosomes always pair. If then sterility is due to the ratio of univalents to bivalents, the F_2 individuals would never be more sterile than the F_1.

On the other hand, it is probable that more than the mere numerical relations of the chromosomes is involved in the sterility of wheat hybrids. The great differences in morphological and physiological characters indicate that the chromosomes of the various species are unlike. The different wheat groups have existed for long periods of time, apparently without the formation of intermediate types, so that the chromosome constitutions may differ greatly even though the polyploid species have originated by reduplication of the diploid set of a primary species. In the pentaploid F_1 hybrid the 14 chromosomes of the Emmer group presumably pair with the allelomorphs of the Vulgare group which they most nearly resemble. The 7 univalents would then perhaps contain factors primarily characteristic of the Vulgare species. If we assume that the members of the bivalent chromosomes can be interchanged in most cases without causing non-functional chromosome combinations, most of the sterility will be caused by the abnormal behavior of the univalent chromosomes. Gametes with 14 chromosomes, and with perhaps 1 or 2 additional univalents, would be fertile and would carry an excess of Emmer factors, while gametes with 21, 20 or 19 chromosomes would carry largely Vulgare factors. The assumption that gametes with 3 or 4 univalents do not develop is in accord with the cytological finding of KIHARA in F_3 segregates. The fertility increases as the chromosome number progresses from 38 to 42. Some 28-chromosome segregates were highly sterile. This latter case may be due to incompatible relations of the members of the 14 bivalent chromosomes. If therefore chromosome mixtures in the bivalents sometimes disturb gametic development,—although in most cases normal development is correlated with an increase or decrease in number of univalent chromosomes, with greatest fertility in 28- and 42-chromosome individuals,—then most of the data both on character transmission and on sterility can be explained. The union of similar gametes would produce segregates with a predominance of either Emmer or Vulgare characters. Intermediates would be formed by a union of the more extreme types. As a result there would be partial linkage of Emmer characters and of Vulgare characters, although pure parental types would rarely be recovered. A preliminary examination of the genetic data indicates that in the F_2 segregates of an Emmer-Vulgare cross many characters are partially linked. It would be possible only in rare cases therefore to combine the Emmer and Vulgare characters in a fertile homozygous individual. In order to explain why varieties or species with intermediate chromosome numbers such as 34 or 36 are not found, it is necessary to assume further that even the bivalent chromosomes cause sterility unless a complete set is present.

If a complete set of chromosomes is essential not only for gametic but also for somatic development, the greater sterility in F_2 could be explained on the ground that plants without a complete set of diploid or triploid chromosomes vary directly in vegetative development with the completeness of chromosome constitution. In the F_1 two complete sets of chromosomes are present, so that somatic development is normal or is even increased through heterosis. In the F_2 the absence of certain chromosomes may result in various degrees of vegetative development, from plants that do not pass the rosette stage to plants which head out but are poorly developed. *In F_1 sterility is apparently due only to gametic chromosome combinations but in F_2 individuals a weak somatic development would prevent gamete formation although such formation might be possible on a normal plant. Thus the greater sterility in F_2 can be attributed, not to greater gametic sterility, but to a combination of somatic and gametic functions.* The fact that there is a rather high degree of negative correlation between sterility and size of plant indicates that much of the F_2 sterility is due to unfavorable vegetative development of the F_2 individuals.

The assumption that gametes are formed with a predominance of Emmer or of Vulgare factors in the F_1, would explain the absence of homozygous segregates combining the desirable characters of the two different groups. Many hybrids between members of the Vulgare and Emmer group have been made, but few if any of the segregates have contained the desired combinations of parental characters in a homozygous condition.

The above explanation would also agree with the hereditary behavior in F_2.[1] We would expect segregates to form in 3 classes, those with predominance of Emmer characters, those with predominance of Vulgare characters, and those with union of the two types. The latter class of segregates might be considerably reduced by sterility. In the F_2 we actually find considerable linkage of characters. In the F_2 of Amby × Kubanka the type of culm, whether solid or hollow, is partially correlated with length of glume, compactness of spikelet, carination of glume, glume color, pubescence, compactness of head, color of grain and texture of grain. It appears that the 17 characters described can be classed in 3 or 4 partially linked groups. Similar relations are found in other crosses. For instance in a cross of Marquis × Alaska with hollow and pithy straw and hard and soft grain, respectively, there is a high degree of correlation between type of culm and hardness of grain ($r = .48 \pm .04$). It is possible, of course, that the high degree of association of parental characters found in these species crosses is due to chromosome linkage or in some cases to multiple effects of a single factor, but crosses within the Vulgare group have shown only a few linked characters. In either case the association of parental characters indicates the difficulty in combining the desirable characters of the Emmer and Vulgare groups.

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[1] The genetic results here summarized were obtained from an investigation in progress by Dr. GAINES and the writer. Five partially sterile hybrids were grown in the F_2 with a total of 714 individuals. The characters of the F_2 individuals were described and the data were punched on cards and analyzed with the aid of a sorting machine. In two crosses, involving descriptions of 17 characters, each character was correlated with every other character to determine the correlation between the various characters and the correlation of characters with sterility.

The correlation between sterility, as indicated by grains per spikelet, and the number of heterozygous factors in F_2 individuals, should be relatively high if the F_1 gametes are predominantly Emmer or Vulgare in functional composition. Two classes of gametes, consisting largely of factors from either parent, would also be found if EAST'S (1915) hypothesis (B) is used to interpret the results. In either case the general method of gamete function is essentially the same, and in the F_2 the segregates resembling the parents should be relatively fertile while those intermediate like the F_1 should be relatively sterile. The correlation between sterility and number of heterozygous factors in an F_2 of *T. vulgare* × *T. turgidum* was found to be practically zero ($r = .03 \pm .06$). Similar results were obtained in other partially sterile wheat hybrids. If most of the 17 characters analyzed are dependent on factors in 2 or 3 chromosomes, little or no correlation would be expected between sterility and number of heterozygous factors.

Although many of the cytological and genetic facts are in accord with the sterility hypothesis presented, more work is necessary to put the chromosome and sterility relationships on a sound basis. Especially is more cytological work necessary in analyzing F_2 individuals. KIHARA (1921) has obtained chromosome counts of 8 F_2 plants in a partially sterile wheat cross and finds the chromosome number ranges from 31 to 42, but not in the frequency that would be expected. KIHARA believes that in the formation of an F_2 plant at least one gamete must have a complete set of either 14 or 21 chromosomes, but if all combinations of univalents and bivalents in the gametes are viable, as KIHARA apparently assumes, and if there is no selective fertilization, then only about 3 percent of the zygotes would develop. Moreover, if the sterility relationships depend on more than mere numerical ratios, and certain specific chromosome combinations cannot function, the degree of fertility will be greatly decreased. If, for instance, all 14 chromosomes of a 14-chromosome gamete must be from the Emmer parent, as KIHARA apparently assumes, then only one gamete in 16,384 would be functional in the F_1

plant. In any case, KIHARA's hypothesis is not in accord with either the cytological facts or with genetic results.

It appears that sterility in wheat hybrids is caused by other factors than those involved in partially sterile Nicotiana hybrids (EAST 1915). In wheat hybrids the abnormal behavior of chromosomes is associated with sterility, although incompatible chromosome combinations probably are factors as in the case of Nicotiana hybrids.

The origin of tetraploidy and hexaploidy in wheat species

The fact that the gametic chromosome number in the species of wheat are in multiples of 7 suggests that the tetraploid and hexaploid species are derived from a primary diploid species by chromosome reduplication. This view is also supported by the fact that several characters are dependent on 2 or 3 independent factors resulting in F_2 ratios of 15 : 1 and 63 : 1. It is also significant that the nearly related cereals have similar chromosome numbers. Rye has 7 haploid chromosomes and in the genus Avena, species are found with 7, 14 and 21 gametic chromosomes (KIHARA 1919). In other genera with polyploid species the tetraploid forms are in most cases known to have originated by chromosome duplication. In the wheat species and in certain other cases there is a striking correlation between chromosome number and cell size, which would indicate that the higher chromosome numbers are the result of duplication and not transverse fragmentation.

On the other hand there is evidence that does not support the view that tetraploidy and hexaploidy in wheat is actually due to chromosomal duplication. In cases where tetraploidy has occurred under experimental conditions the reduplicated sets of chromosomes assort at random in reduction which is not the case in tetraploid wheat species.

If the Emmer and Vulgare groups are the result of polyploidy, then, most if not all characters should be dependent on multiple factors. Of the characters which have been genetically investigated in wheat, 14 are apparently dependent on 1 factor, 4 characters behave as dihybrids, and only 1 character, red grain color, is clearly due to 3 independent factors in some cases. The latter character may also depend on only 1 or 2 factors in certain varieties. In wheat species, where the chromosomes of the polysomic sets assort in pairs, mutations could result in characters dependent on 1 or 2 factors; but in tetraploid species, where the chromosomes of a tetrasomic set assort at random, a single factor could not exist in the homozygous condition. It is possible that chromosomal duplication occurred very early in the history of cultivated wheat and that in the course of time the individual chromosomes came to differ so that they no longer assorted at random within a reduplicated set.

Representations of all of the three groups of wheat can be traced back to prehistoric times (PERCIVAL 1921). Einkorn was probably one of the chief wheats grown in central Europe in the Neolithic period. Emmer was grown in Europe in prehistoric times and was grown in Egypt as early as 5400 B. C. It was the most important cereal in Egypt from the early ages until supplanted by Durum and Vulgare varieties in the Graeco-Roman period. Although representatives of the Vulgare wheat group were known to exist in Europe in prehistoric ages they were not the chief varieties grown until comparatively recent times. The great age of the three wheat groups may also explain why the groups differ so markedly in morphological characters even if they were originally derived from a single primary species by chromosome duplication. Although the species within each group overlap considerably, the differences between the three sterility groups are rather distinct. PERCIVAL has summarized the important characters which differentiate the Vulgare group from the Emmer group as follows: (1) Differences in arrangement of hairs on the leaf; (2) thin-walled, hollow culms; (3) a tough, non-disarticulating rachis; (4) absence of keel on the lower part of the glume in most cases; and (5) the comparatively short awns of the fully bearded varieties and the occurrence of beardless and semi-bearded varieties. The greatest difference is, of course, the high quality of the gluten in most of the Vulgare varieties. No variety of the Emmer group contains gluten of the quality necessary for the production of light spongy bread. The species of the Emmer group are, in general, best adapted to a hot dry climate, and because of susceptibility to cold weather, they are in few cases sown in the fall in the temperate zone.

Many of the characters which distinguish the Vulgare from the Emmer group are found in *T. aegilops ovata* or *T. aegilops cylindrica* and accordingly PERCIVAL has concluded that the Vulgare group is a hybrid race resulting from an early cross or crosses of members of the Emmer group and *T. aegilops*. The hybrids of the present species of the Emmer group or even *T. dicoccoides* with *T. aegilops* are, however, nearly or quite sterile in the F_1.

Chromosome number and adaptability in wheat species

The characters of the Vulgare group are subject to very wide variation and the great number of varieties and intermediate forms are believed by

PERCIVAL to be due to their hybrid origin. The great variability and adaptability of the Vulgare wheats may, however, be due to other causes. EAST (1915) suggested that variability of a species would be correlated with the chromosome number and DORSEY in 1916 suggested that the adaptability of a species might be correlated with chromosome number. With an increase of chromosome number the number of factorial combinations would increase, thus resulting in a greater range of adaptation. There is certainly a high degree of correlation between chromosome number and adaptability in the species of wheat. The number and distribution of the varieties of the cultivated species of wheat are shown in table 7.

TABLE 7

Number and distribution of varieties of the Einkorn, Emmer and Vulgare groups. (*Data from* PERCIVAL 1921.)

	T. monococcum	*T. dicoccum*	*T. durum*	*T. polonicum*	*T. turgidum*	*T. pyramidale*	*T. vulgare*	*T. compactum*	*T. sphaerococcum*	*T. Spelta*
Number of "varieties"	3	40	22	23	27	5	26	20	6	12
Groups		Emmer					Vulgare			
Haploid chromosome number	7	14					21			
Distribution of "forms"										
S. E. Europe, S. W. Asia	*	10					9			
Southern Europe	*	77					42			
Russia		7					12			
Central and N. Europe		27					102			
England		13					91			
India		2					4			
Australia and New Zealand		3					63			
South Africa		4					23			
Japan and China							11			
North America		7					109			
South America		1					7			
Total		151					536			

*Species of Einkorn grown but no cultivated "forms" described.

PERCIVAL (1921) recognizes only two wheat species, *T. aegilopoides* and *T. dicoccoides*, and suggests that the other types might be classed as "cultivated species." Under the term "forms" PERCIVAL classes what are commonly known as varieties, such as Marquis, Bluestem, Kubanka, etc. It will be seen in table 7 that the number of the varieties is greatest in the Emmer group, but that the Vulgare group contains almost four times as many "forms." The distribution of the different groups is also significant. The members of the Einkorn and Emmer groups are found in greatest numbers in the regions where wheat originated. In general the region has a warm dry climate. The members of the Emmer group are comparatively rare in Australia, England, South Africa, Japan and North America. Members of the Vulgare group are found all over the world wherever wheat is grown and under diverse climatic conditions. Although the Vulgare group is more variable and adapted to a greater range of conditions they are not always the highest yielders. Certain varieties of *T. turgidum* are the most productive of all wheats, and under certain conditions varieties of *T. durum* will outyield the Vulgare varieties. The correlation between chromosome number and adaptability in wheat species may not depend on a causal relationship. The Vulgare group may be more highly selected and more widely distributed because it is the only wheat suitable for making light bread. The greater variation and adaptability would also be expected if the Vulgare group is a vast hybrid race as PERCIVAL has suggested. A study of chromosome number in relation to variations and adaptability in polyploid species under natural conditions would be of interest.

The value of tetraploidy

Since tetraploidy usually results in increased size of plant tissues and possibly permits greater adaptability, the development of tetraploid species may be of value. WINKLER (1916) has obtained tetraploid forms of tomatoes and nightshade in connection with his work on graft hybrids. The tetraploid forms were larger than diploid forms but unfortunately they were sterile. A number of investigators have been able to induce changes in the chromosome number of somatic cells by treatment with various reagents (SAKAMURA 1920) but it appears to be questionable if such changes can be maintained and established in later generations. CASTLE (1921) has suggested that the French prune mutation described by SHAMEL (1919) is the result of tetraploidy. It is not improbable that the best cases of bud mutations involving increased productivity (if such mutations actually occur) will be found to be associated with tetraploidy or chromosome duplication.

DESCRIPTION OF PLATES

Preparations from pollen mother cells at the time of the reduction divisions. The drawings were made with the aid of a camera lucida. All figures were drawn from single sections. Magnification 750 diameters. No reduction. The reproduction of these drawings has been made possible by financial assistance from BUSSEY INSTITUTION.

PLATE 1

Triticum monococcum

FIGURE 1.—The 7 paired chromosomes at diakinesis.
FIGURE 2.—Metaphase, polar view. Heterotypic division.
FIGURE 3.—Metaphase, side view.
FIGURE 4.—Late anaphase, 7 chromosomes at each pole.
FIGURE 5.—Polar view of late anaphase showing the 7 chromosomes split for the homoeotypic division.
FIGURE 6.—Telophase of the homoeotypic division.

The Emmer group

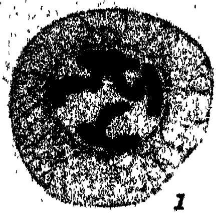
FIGURE 7.—Diakinesis. 14 pairs of chromosomes. *T. durum.*
FIGURE 8.—Metaphase, polar view. 14 chromosomes.

T. polonicum

FIGURE 9.—Metaphase, polar view. *T. durum.*
FIGURE 10.—Metaphase, side view. *T. polonicum.*
FIGURE 11.—Anaphase of heterotypic division. *T. polonicum.*
FIGURE 12.—Interkinesis. Resting nuclei. *T. polonicum.*
FIGURE 13.—Telophase of homoeotypic division. *T. durum.*

The Vulgare group

FIGURE 14.—Metaphase, polar view. 21 chromosomes. *T. Spelta.*
FIGURE 15.—Metaphase, polar view. 21 chromosomes. *T. vulgare.* (Preston)
FIGURE 16.—Metaphase, side view. *T. vulgare.* (Preston)
FIGURE 17.—Late anaphase of heterotypic division, side view. *T. vulgare.* (Preston)
FIGURE 18.—Late anaphase, polar view. *T. vulgare.* (Preston)
FIGURE 19.—Telophase. *T. vulgare.* (Marquis)
FIGURE 20.—Metaphase. Homoeotypic division, polar view. *T. vulgare.* (Amby)
FIGURE 21.—Telophase, homoeotypic division. *T. vulgare.* (Amby)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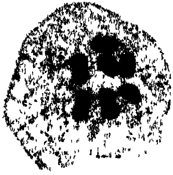
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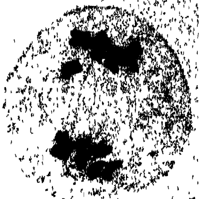
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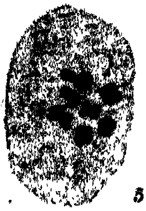
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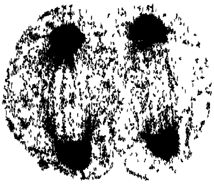
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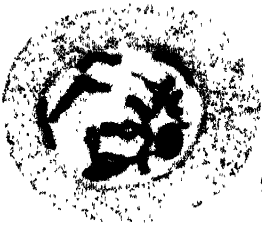
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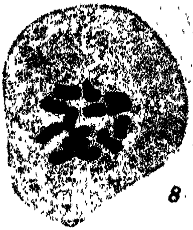
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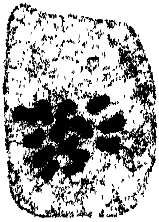
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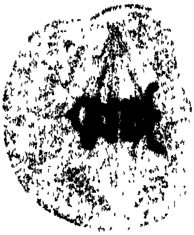
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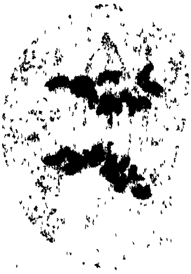
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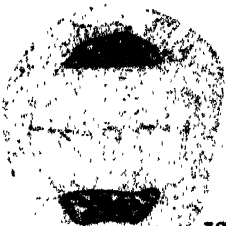
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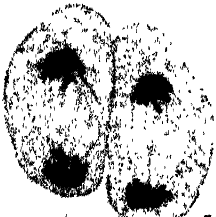
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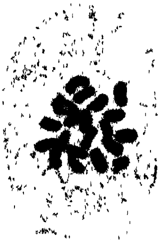
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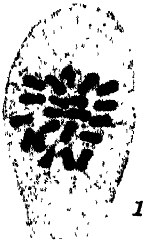
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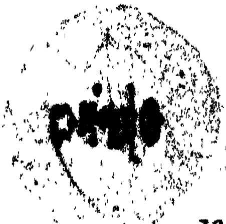
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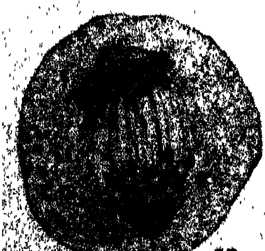
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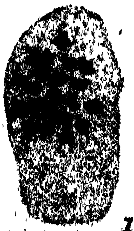
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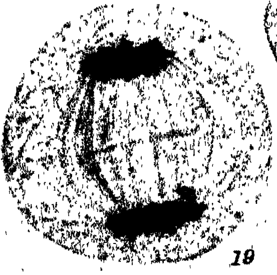
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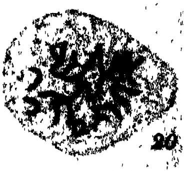
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H. J. S. and K. S.

The value of polyploidy may have certain limitations. As TUPPER and BARTLETT (1918) have suggested, a few doublings in chromosome number would increase the size of the cell so that respiration and nutrition would be retarded. In Crepis and Chrysanthemum, however, the chromosome number has apparently been duplicated many times without disastrous results. MULLER (1918) believes that tetraploidy would hinder evolution because recessive mutants could rarely manifest themselves. If recessive factors have some effect in the presence of dominant factors, which is frequently or perhaps usually the case, then increased variability would result even if the pure recessive could rarely be isolated.

There has been considerable discussion as to the origin of tetraploid species especially in Oenothera. GATES (1915) argues that *Oe. gigas* must have originated by a doubling of chromosomes at an early stage in somatic development of a diploid species, because of the rare occurrence of diploid gametes. This interpretation is undoubtedly the most reasonable in cases where tetraploid individuals have suddenly originated from a diploid species. If a diploid gamete united with a normal haploid gamete and the chromosomes in the trisomic sets assorted at random 25 percent of the F_2 segregates would be tetraploid. The occasional occurrence of diploid gametes has been observed in several diploid species and would afford an opportunity for the formation of tetraploid species although the latter would originate from a triploid individual and not directly from the original diploid species.

Pollen-grain variability

The pollen of F_1 plants of species hybrids is much more variable than the pollen of pure species.

The remarkable relation between heterosis or degree of germinal mixture and variability of pollen grains is especially interesting because the pollen grains belong to the gametophytic generation. In most cases recorded the characters of the pollen grain are determined by the mother plant. BATESON (1909) has found that the shape and color of pollen in the sweet pea behave as sporophytic characters. EAST (1916) has found a similar behavior of pollen in Nicotiana and also finds that self-sterility behaves as a sporophytic character (EAST 1919). On the other hand BELLING (1914) has found segregation of normal and aborted pollen grains in semi-sterile F_1 hybrids, and PARNELL (1921) finds segregation of equal numbers of starchy and glutinous pollen grains in an F_1 rice hybrid. The occurrence of the starchy and glutinous pollen grains

DESCRIPTION OF PLATE 2

T. monococcum (7-chromosome gametes) × *T. turgidum* (14-chromosome gametes)

Figure 22.—Metaphase of heterotypic division with 7 bivalents (1 divided) and 7 univalents at the poles.

Figure 23.—Late anaphase with approximately 10 chromosomes at either pole due to the division of the bivalents and the random assortment of the univalents without dividing.

Figure 24.—Early telophase. Split chromosomes, originally members of the bivalent chromosomes, just reaching the poles.

Figure 25.—Telophase. No lagging chromosomes.

Figure 26.—Anaphase of homoeotypic division. Vulgare group (21-chromosome gametes) × Emmer group (14-chromosome gametes), F_1 plants.

Figure 27.—Metaphase, polar view, of homoeotypic division with about 10 chromosomes in each figure.

Figure 28.—Metaphase of heterotypic division, polar view. Fourteen bivalents and 7 univalents. Amby × Kubanka.

Figure 29.—Metaphase showing bivalents and univalents. Bluestem × Alaska.

Figure 30.—Metaphase of heterotypic division showing irregularly distributed univalents. Note the attachment of the spindle fibers and apparent tension of the chromosomes. Amby × Kubanka.

Figure 31.—The bivalents have divided leaving the 7 univalents and apparently several additional unpaired chromosomes on the equatorial plate. Amby × Kubanka.

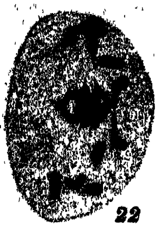
Figure 32.—The 14 bivalents have divided and reached the poles while the 7 univalents are dividing equationally. Amby × Kubanka.

Figure 33.—A similar stage in Bluestem × Kubanka.

Figure 34.—A similar stage in Bluestem × Kubanka.

Figure 35.—The 7 univalents have divided and have nearly reached the poles. Bluestem × Alaska.

Figure 36.—Homoeotypic division showing univalents which do not divide but pass at random to either pole and join the original 14 bivalents.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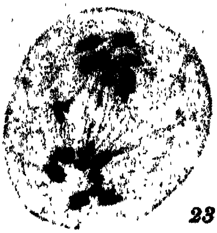
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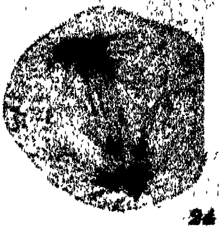
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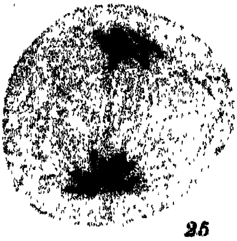
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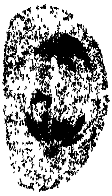
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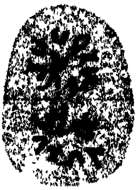
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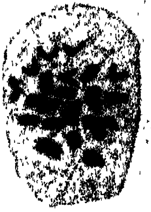
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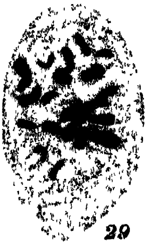
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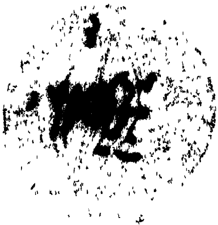
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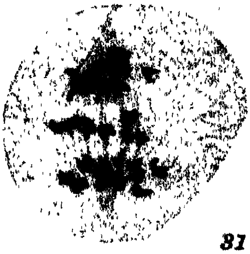
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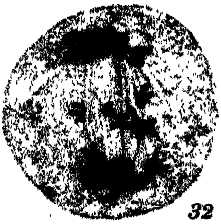
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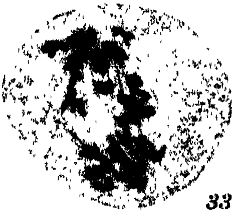
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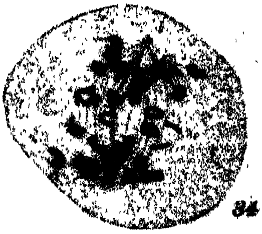
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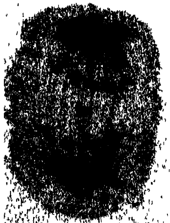
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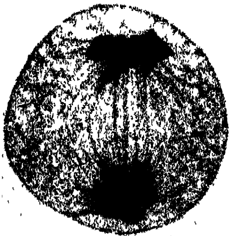
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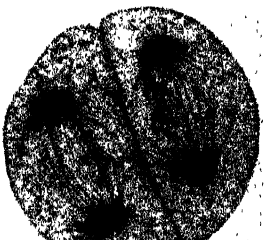
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H. J. S. and K. S.

is undoubtedly due to the effect of the segregated factors. In the wheat hybrids the factors for growth apparently segregate into various proportions of maternal and paternal factors in the F_1 and the various combinations of these factors may cause increasd variation in size of pollen grains, ranging from completely aborted grains to those much larger than normal. The increased variability is due here to combinations of nonhomologous chromosomes acting in the gametophytic generation and to differences in chromosome number. In cases where the gametophytic characters are controlled by the sporophyte the characters may be those primarily caused by the sporophyte, such as pollen-grain shape and possibly color. In other cases certain characters, such as growth and starch-formation, may be controlled by the gametophytic chromosomes at an early stage in the development of the gametophyte, while perhaps other characters cannot be brought into expression until the necessary development of the organism has been attained.

SUMMARY

The chromosome number and behavior has been determined for the cultural species of Triticum and for certain partially sterile hybrids.

The gametic chromosome number is 7 for *T. monococcum*; 14 for the Emmer group, consisting of *T. dicoccum*, *T. durum*, *T. polonicum*, and *T. turgidum;* and 21 for the Vulgare group, consisting of *T. vulgare*, *T. compactum* and *T. Spelta*. Rye (*Secale cereale*) has 7 gametic chromosomes.

In the F_1 hybrid of *T. monococcum* × *T. turgidum* 7 chromosomes are contributed by one parent and 14 by the other parent. In the heterotypic division of the pollen mother cells there are 7 bivalents and 7 single chromosomes. The 7 bivalents divide normally, but the 7 univalents pass at random to either pole without dividing. In the homoeotypic division there are usually about 10 or 11 chromosomes which apparently divide normally in most cases. Tetrads and one-nucleate pollen grains appear to be normal, but very few normal mature pollen grains are formed.

In F_1 hybrids between members of the Emmer group and members of the Vulgare group, 14 chromosomes are contributed by one parent and 21 by the other parent. In the first meiotic division there are 14 bivalent and 7 univalent chromosomes. The bivalents divide normally, but the univalents do not become oriented on the equatorial plate until the bivalents have divided. The 7 lagging univalents divide equationally and 7 chromosomes pass to each pole. In the homoeotypic division the

DESCRIPTION OF PLATE 3

Micro-photographs of chromosomes in the reduction divisions of the pollen mother cells in wheat species, rye, and wheat hybrids. Photographs not retouched.

FIGURE 37.—Late anaphase showing the 7 chromosomes in Einkorn.

FIGURE 38.—Late anaphase, side view. Einkorn.

FIGURE 39.—Late anaphase with 14 chromosomes at one pole. Polish.

FIGURE 40.—Metaphase, polar view. 21 chromosomes. Preston.

FIGURE 41 A, B.—Diakinesis in rye, showing the 7 pairs of twisted chromosomes.

FIGURE 42.—Metaphase showing univalents and bivalents in (Amby × Kubanka) F_1.

FIGURE 43.—Bivalent chromosomes have divided and reached the poles while the split univalents are on the heterotypic plate. (Amby × Kubanka) F_1.

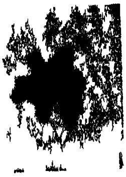
FIGURE 44.—Metaphase, anaphase, and telophase in 3 adjacent pollen mother cells in an anther of Preston.

FIGURE 45.—Same as figure 43.

FIGURE 46.—Telophase, no lagging chromosomes. (Amby × Kubanka) F_1.

FIGURE 47.—Metaphase, side view of the homoeotypic division showing several irregularly distributed chromosomes. (Amby × Kubanka) F_1.

FIGURE 48.—Homoeotypic division showing lagging chromosomes. Same as figure 36. Amby × Kubanka) F_1.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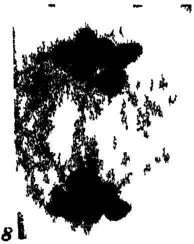
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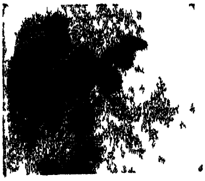
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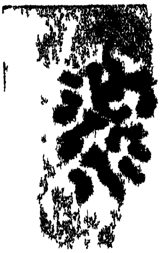
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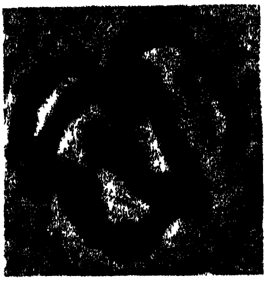
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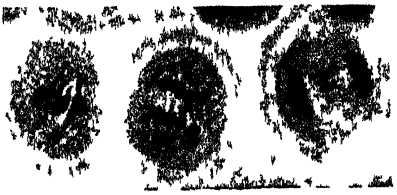
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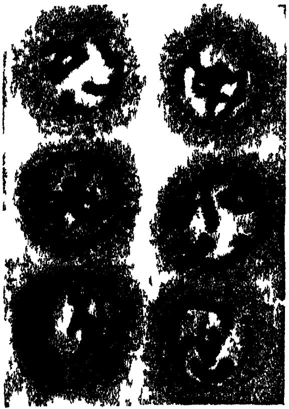
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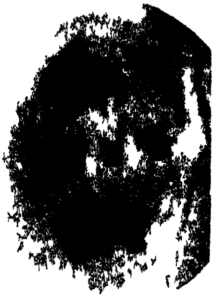
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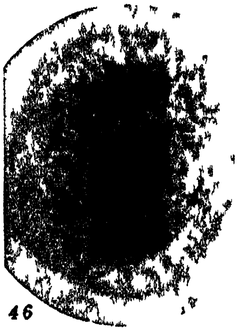
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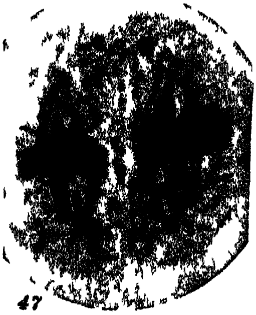
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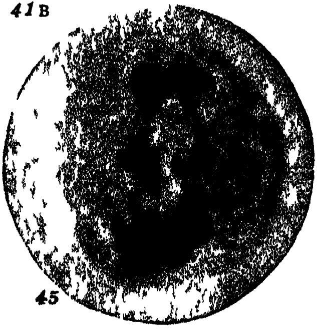
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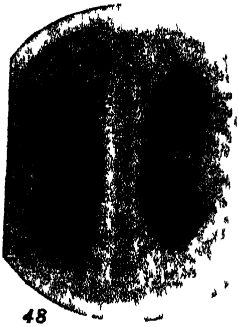
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J. W. B. and K. S. photo.

original members of the bivalents divide normally, but the remaining 7 chromosomes pass at random to either pole without dividing. As a result the chromosome number of the microspores varies from 14 to 21. The tetrads and one-nucleate pollen grains appear to be normal, but in later stages about 20 percent of the pollen grains are obviously imperfect and undoubtedly a larger percentage is non-functional.

The size of the pollen grains is closely correlated with the chromosome number in the various species of wheat.

The pollen grains of fertile species hybrids are more variable than the pollen grains of the parental species due to various degrees of compatibility of the combinations of non-homologous chromosomes in the gametophytic generation. In partially sterile hybrids where the parental species differ in chromosome number, the pollen grains are extremely variable, due to difference in chromosome number and to more or less compatible chromosome combinations.

The sterility in the hybrids described can be accounted for on a hypothesis involving, (1) the numerical or unbalanced relations of the chromosomes resulting from the irregular meiotic divisions, and (2) the specific interrelations of the parental chromosomes. In the numerical relations the development of gametes varies as the chromosome number approaches the normal gametic number (7, 14 or 21). It is assumed that the greater the gametic chromosome number the greater can be the deviation from the normal number. The specific relations of the gametic chromosomes will depend on the extent that chromosomes from one parent can be substituted for those of the other parent. Gametic perfection will vary as the chromosome constitution approaches that of the parental forms. The 7 univalents in the Emmer-Vulgare F_1 hybrids presumably carry most of the factors which differentiate the Vulgare characters from the Emmer characters.

This hypothesis will explain (1) the differences in sterility of the various species hybrids, (2) the partial association of the original parental characters in the F_2 segregates, (3) the absence of varieties or species with intermediate chromosome numbers, and (4) the difficulty in obtaining homozygous segregates combining the desirable characters of the parental species in partially sterile wheat hybrids.

In all cases the F_1 plants are unusually vigorous and sterility is not due to poor vegetative development, but is caused by the formation of non-functional gametes. Sterility in F_2 segregates may be greater than in F_1 individuals due, not to greater gametic sterility *per se*, but to a combination of weak somatic development and gametic sterility.

The evidence for and against the occurrence of chromosome duplication in wheat species is presented and discussed.

There is a high degree of correlation between chromosome number and adaptability in the wheat species, but the relation may not be a causal one.

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STERILITY IN WHEAT HYBRIDS. III. ENDOSPERM DEVELOPMENT AND F_2 STERILITY

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Crosses of wheat species with different chromosome numbers result in small or wrinkled seeds. These seeds consist of the F_1 endosperm and the embryo of the F_1 plant. Although the F_1 endosperm is usually poorly developed, the F_1 plant is even more vigorous than the parents. The F_1 plants are partially sterile and bear grains (F_2 endosperms) which vary greatly in size. Since small wrinkled grains are characteristic of partially sterile hybrids the small wrinkled grains borne on the F_1 plant might be expected to produce partially sterile F_2 plants, while the large plump grains would produce relatively fertile F_2 individuals.

It is well known that wide species crosses in wheat result in partially sterile F_1 plants and that in F_2 all degrees of sterility are found, ranging from plants which never pass the rosette stage, to those quite as fertile as the parents. A study of sterility in view of the chromosome relationships should be of value.

The parental varieties of wheat used were *Triticum durum* Desf. (Kubanka) and *T. vulgare* Vill. (Pacific Coast Bluestem). The crosses were made in 1916 and the F_1 was grown at BUSSEY INSTITUTION in 1917. The F_2 was grown in the greenhouse in 1917–1918.

WEIGHT OF SEEDS OF PARENTS AND HYBRIDS AND F_1 STERILITY

The degree of sterility, as indicated by the grains set per spikelet, and weight of grains of parents and the F_1 hybrids are shown in table 1. The F_1 plants are about one-fourth as fertile as the parents, although they are more vigorous vegetatively as indicated by the greater number of heads per plant. There is little difference in the fertility of the reciprocal hybrids. For the 30 F_1 plants the average number of grains per spikelet was found to be $0.57 \pm .03$ with a standard deviation of $0.25 \pm .02$. The sterility of the F_1 plants varied from .07 to 1.00 grain per spikelet. It appears probable that with larger numbers some F_1 individuals would

GENETICS 7: 553 N 1922

TABLE 1

Weight of grain and sterility in parents and hybrids.

NO. OF PLANTS	NO. OF HEADS	NO. OF SPIKELETS	NO. OF GRAINS	GRAINS PER SPIKELET	WEIGHT OF GRAINS IN 5-MG UNITS															NUMBER	MEAN	STANDARD DEVIATION
					2.5-7.5-	7.5-	12.5-	17.5-	22.5-	27.5-	32.5-	37.5-	42.5-	47.5-	52.5-	57.5-	62.5-	67.5-	72.5-77.5-			
					Kubanka																	
6	9	144	386	2.68					1	8	25	30	45	55	89	82	20	1		356	48.9±.3	8,9±.2
					Bluestem																	
7	23	354	789	2.23				1	2	14	27	51	115	123	100	24	2			458	45.2±.2	7,4±.2
					(Kubanka × Bluestem)F₁ endosperm																	
							2	4	7	6	4	1								24	24.4±.9	6.4±.6
					(Kubanka × Bluestem)F₁ (F₂ endosperm)																	
15	152	2323	1413	.61	4	19	31	50	106	124	138	154	183	161	175	121	103	33	11	1413	41.4±.3	14.5±.2
					(Bluestem × Kubanka)F₁ (F₂ endosperm)																	
15	131	1932	990	.51	9	22	34	50	77	109	115	123	114	110	95	70	37	19	6	990	38.1±.3	14.7±.2

be totally sterile, although the percentage of such plants would be small. The variation in sterility of the F_1 plants is apparently not due to differences in vegetative development, because the correlation between sterility, as indicated by the number of grains set per spikelet, and vegetative vigor, as indicated by the number of heads per plant, is practically zero ($r = -.08 \pm .12$).

Kubanka has somewhat larger seeds than Bluestem, but the variability of grain weight does not differ greatly although the difference is statistically significant. The grains resulting from the immediate cross (F_1 endosperm) are but little more than half as heavy as the grains of the parents and are no more variable. The grains borne by the F_1 plants[1] (F_2 endosperm) are extremely variable, with a standard deviation of nearly twice that of the parental grain weight. The average grain weight of the F_1 plants is greater when Kubanka is used as the female parent, but the difference, although statistically significant, may depend on environmental and not genetic factors.

STERILITY IN THE F_2 GENERATION

The seed from an F_1 plant of Kubanka × Bluestem was planted in the greenhouse under very uniform and favorable conditions. The F_1 plant selected was representative of all F_1 plants, both in vegetative development and sterility. Of the 84 seeds planted 6 failed to germinate, 78 seeds germinated but of these only 61 produced heads and only 47 produced grain. In the entire F_2 only 1 or perhaps 2 plants were as fertile as the parents. The descriptions of the F_1 grain (F_2 endosperm) and the resulting F_2 plants are given in table 2.

The sterility in F_2 ranged from plants which were totally sterile to one which set 2.66 grains per spikelet. The mean sterility of the 61 plants that headed was found to be .83 with a standard deviation of .64. Thus in the F_2 a larger proportion of plants is totally sterile than in the F_1. Moreover, many F_2 plants fail to develop normally and a large percentage fails to produce heads. Under field conditions probably half of the F_1 grain fails to produce viable F_2 plants, and under unfavorable climatic conditions nearly all F_1 grains fail to develop, even though standard varieties may show more than 50 percent germination under the same conditions. Much of the extreme sterility in F_2 is undoubtedly due to

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[1] The mean weight and variability of seeds of parents and F_1 plants were relatively uniform, so all individuals of each class were grouped together. The variability in seed weight is not due to differences in average seed weight of individual plants.

TABLE 2

Kubanka × Bluestem—F_1 *and* F_2.

(*Greenhouse* 1917.)

F_1			F_2				F_1			F_2			
Grain No.	Weight in mg*	Wrinkled or plump	Height of plant in cm	No. of spikelets	No. of grains	Grains per spikelet	Grain No.	Weight in mg	Wrinkled or plump	Height of plant in cm	No. of spikelets	No. of grains	Grains per spikelet
1	15	W	127	120	156	1.30	43	45	P	96	54	62	1.15
2	20	W					44	45	P	128	80	20	.25
3	20	W					45	45	P	128	60	45	.75
4	20	P					46	45	P	117	+	0	.00
5	25	W	132	106	142	1.34	47	45	P	*9*			
6	25	W	131	90	111	1.23	48	45	P	137	148	213	1.44
7	25	W	118	72	78	1.10	49	50	W	145	66	21	.32
8	30	W	123	68	23	.34	50	50	W	109	+	0	.00
9	30	W					51	50	W	128	+	0	.00
10	30	W	124	100	200	2 00	52	50	W	54	+	0	.00
11	30	P	136	115	58	.50	53	50	W	140	100	41	.41
12	30	P					54	50	W	*14*			
13	35	W	119	92	127	1 38	55	50	W	*13*			
14	35	W	115	124	177	1 42	56	50	P	107	88	16	.18
15	35	W	130	74	6	.08	57	50	P	80			
16	35	W	128	47	125	2.66	58	50	P	30			
17	35	W	130	150	68	.45	59	50	P	115	+	0	.00
18	35	P	105	74	10	.14	60	50	P	40			
19	40	W	126	116	152	1.31	61	50	P	129	188	391	1.81
20	40	W	136	102	96	.94	62	50	P	*13*			
21	40	W	*10*				63	55	W	*14*			
22	40	W	122	122	185	1.52	64	55	W	115	151	253	1.70
23	40	W	35				65	55	W	142	102	37	.37
24	40	W	*10*				66	55	W	114	86	118	1 37
25	40	W	110	60	77	1.28	67	55	W	*14*			
26	40	W	105	80	57	.71	68	55	P	124	138	180	1.30
27	40	W	40				69	55	P	105	100	131	1.31
28	40	W	*11*				70	55	P	87	+	0	.00
29	40	W					71	55	P	147	36	52	1.44
30	40	W	127	130	183	1.41	72	55	P	145	192	306	1.60
31	40	P	106	112	73	.65	73	55	P	50	+	0	.00
32	40	P	*7*				74	60	W	130	94	111	1.18
33	40	P	100	+	0	.00	75	60	W	115	+	0	.00
34	40	P	117	80	15	.19	76	60	P	60			
35	40	P	60	+	0	.00	77	60	P	135	126	216	1.71
36	40	P	40				78	60	P	86	+	0	.00
37	45	W	132	70	50	.70	79	60	P	153	104	136	1.31
38	45	W	121	68	78	1.14	80	65	W	90	+	0	.00
39	45	W	100	130	59	.45	81	65	W	115	100	167	1.67
40	45	W	137	126	67	.53	82	65	W	155	34	10	.30
41	45	W	108	100	29	.29	83	65	P	61	+	0	.00
42	45	P	130	84	138	1.64	84	70	W	63	+	0	.00

poor vegetative development of certain plants. Such a relation is indicated by the relatively high correlation between sterility and height of the 61 F_2 plants which headed ($r = .42 \pm .07$). Among the 47 plants which set grain the high sterility of certain individuals does not seem to be due to poor vegetative development, since there is no significant correlation between height and sterility of the 47 F_2 plants ($r = .04 \pm .10$).

The 84 F_1 grains were extremely variable in weight and were also variable in shape,—whether wrinkled or plump. In table 2 the F_1 grains are arranged in order of weight and arbitrarily classified as wrinkled or plump. The average weight of the wrinkled grains was found to be but little less than the weight of the plump grains. The correlation between weight and plumpness of grain was found to be rather low ($r = .23$). The correlation was determined by a method suggested by PEARSON (1909).

Among the F_1 seeds which failed to germinate, a majority are in the lower weight classes, but among the 78 plants which grew there is little or no correlation between weight of F_1 grains and height of the resulting F_2 plant ($r = -.10 \pm .05$). The correlation is not greatly increased by using only the 61 F_2 plants which headed ($r = -.20 \pm .08$.) The size of the F_1 grain gives little indication of the probable size of the F_2 plant. The plumpness of F_1 grains has little or no effect on the size of F_2 plants. For the 78 F_2 plants the correlation between wrinkled or plump F_1 grain and height of F_2 plants was found to be only .10, and for the 61 plants which headed, $r = .10$.

A similar correlation was found between F_1 grain weight and F_2 sterility. For the 47 F_2 plants that headed, the correlation between F_1 grain weight and F_2 sterility was $.03 \pm .10$, and for all 78 plants, $r = -.19 \pm .08$. The correlation between wrinkled or plump F_1 grains and F_2 sterility was found to be .24 for the 61 F_2 plants which headed. Although small wrinkled grains are characteristic of partially sterile hybrids there is little or no correlation between F_1 grain weight or shape and the height or sterility of the resulting F_2 plants.

DISCUSSION AND SUMMARY

In a cross of Kubanka × Bluestem a male gamete with 21 chromosomes unites with an egg cell containing 14 chromosomes. In the endosperm there are 28 chromosomes of maternal origin and 21 chromosomes of paternal origin while in the reciprocal cross 42 chromosomes are contributed by the polar nuclei and only 14 by the sperm nucleus. Such unbalanced conditions of the chromosome sets might be expected to result in abnormal endosperm development, although in pure species the triploid condition of the endosperm does not result in abnormal development, unless endosperm formation itself is considered abnormal. Endosperm formation is not due simply to the triploid condition, however, because triploidy is found in the vegetative cycle of a number of plants.

The greatly increased variability of the F_1 grain (F_2 endosperm) can be attributed to differences in chromosome number, to recombinations of factors for growth, and to more or less compatible chromosome combinations. Although the maximum mixture of parental chromosomes is found in the F_1 endosperm the F_2 endosperms may in many cases be significantly smaller than F_1 endosperms, due to inharmonious chromosome relations.

Since poorly developed F_1 endosperm is associated with sterility and unusual vegetative vigor of the F_1 plant, a high degree of correlation would be expected between F_2 endosperm development and sterility and vegetative development of F_2 plants. An analysis of the data shows, however, that there is little or no correlation between F_2 endosperm development and F_2 sterility or vegetative development. Apparently the results of the peculiar chromosome relationships in endosperm formation do not indicate the nature of the chromosome relations of the accompanying embryo. This relation may be due to the greater opportunity for an unbalanced condition of maternal and paternal chromosomes in the endosperm.

The increased sterility in F_2 individuals as compared with the F_1, and especially the weak somatic development of many plants, may be attributed to chromosome combinations incompatible for vegetative development. The chromosome compatibility may depend on numerical or balanced relation of the chromosomes or on specific chromosome relationships. In F_1 plants two complete sets of chromosomes are present and the F_1 plant is unusually vigorous even though it is partially sterile. In F_2 individuals a complete set of chromosomes of either parent would rarely be found and the perfection of somatic development would be expected to vary with the completeness of the parental sets of chromosomes. Thus in F_2 many individuals are completely sterile because of weak vegetative development as well as gametic sterility.

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INHERITANCE OF ZIGZAG CULMS IN MAIZE

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INTRODUCTION

Maize plants with zigzag culms were first observed by Dr. R. A. EMERSON in a number of fourth-generation families from a cross between Tom Thumb pop corn and a Missouri dent corn. The appearance of plants with zigzag culms was probably due to a recombination of factors from the plants in the original cross. In the summer of 1917 Dr. EMERSON turned over to me for further study a strain of maize breeding true for zigzag culms. In a preliminary paper (EYSTER 1920) it was suggested that two factors are concerned in the production of zigzag culms in maize. When both factor pairs are recessive the culms of the main plant and tillers are zigzag, but the dominant allelomorph of either pair is sufficient to produce normal culms. Sufficient data have since been accumulated, it is thought, to establish this explanation. Much of the experimental work reported here was done at CORNELL UNIVERSITY. I am indebted to that institution and to DR. EMERSON, especially, for placing at my disposal facilities for the work. The remainder was done at the MISSOURI AGRICULTURAL EXPERIMENT STATION, as a project of the Department of Field Crops, UNIVERSITY OF MISSOURI. I am also indebted to L. A. EYSTER for taking some of the notes.

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DESCRIPTION OF ZIGZAG CULMS

Emerson (1912) described maize plants with zigzag culms in connection with a number of other recessive abnormalities in maize which he was studying at that time. The writer (Eyster 1920) more recently published a series of illustrations to show the nature of zigzag culms and the variation in its expression.

Zigzag culms come into expression at about the time the tassel and ear shoots make their appearance. The first evidence is an apparent flattening and widening of the culm. This is due to the spreading apart of the over-lapping leaf sheaths by the crooks in the culm. In extreme cases the sheaths are pushed aside so far that they no longer clasp the culm and therefore give no mechanical support to the rapidly elongating culm.

When the leaf sheaths are removed it is seen that some of the internodes, varying from two or three in some plants to as many as ten or twelve in other plants, are shortened, thickened and crooked. It is usually the internodes in the ear-shoot region that are so affected. Sometimes the abnormality appears in more than one region of the culm with apparently normal internodes between the affected regions. Plants in which a large number of internodes are abnormal are very much dwarfed, being in some instances less than two feet tall. Plants having a smaller number of abnormal internodes approach the normal plants in height and appearance.

Maize, like other monocotyledonous plants, has a region of meristematic cells at the base of each internode. The abnormal internodes are such as would be produced by a more rapid growth in the meristem on the side of the internode of the leaf attachment.

TERMINOLOGY

$Z_g\, z_g$,—a factor pair for zigzag culms in maize. This factor pair is linked with the pair $G_s\, g_s$.

$Z\, z$,—a second factor pair for zigzag culms.

$G_s\, g_s$,—a factor pair for green-striped leaves (Lindstrom 1918).

INHERITANCE OF ZIGZAG CULMS

Behavior in the F_1 and F_2 generations

Plants with zigzag culms breed true when self-pollinated. When plants with zigzag culms are crossed with plants with normal culms, the F_1 offspring have normal culms.

Eleven F_2 progenies are listed in table 1. These progenies fall into two well defined groups. The one group has normal and zigzag plants in ratios approaching 15 : 1 and the other group has normal and zigzag plants in ratios approaching 3 : 1. The ratios of the first group vary from 13 : 1 to 19 : 1; those of the second group from 2.7 : 1 to 3.1 : 1. The results are in accord with the interpretation that culm form is determined by two independent factors.

TABLE 1

F_2 progenies of the cross normal × zigzag.

PEDIGREE NUMBERS	NORMAL CULMS	ZIGZAG CULMS	RATIO
Dihybrid			
535	343	23	14.9 : 1
918	207	16	13.0 : 1
919	186	13	14.3 : 1
1525	323	19	17.0 : 1
1526	147	10	14.7 : 1
1530	259	19	13.6 : 1
1531	308	18	17.1 : 1
1532	380	20	19 0 : 1
Total	2153	138	16.2 : 1
Monohybrid			
1527	291	93	3 1 : 1
1528	290	107	2.7 : 1
1529	178	62	2.9 : 1
Total	759	262	2 9 : 1

In crossing zigzag plants with normal plants from various sources it might be expected that some of the crosses would involve both factors for zigzag culms, and others only one. When both factors are involved a 15 : 1 F_2 ratio is expected, and when only one factor is involved a 3 : 1 F_2 ratio. The observed results closely approximate the expectation in both cases. A comparison of the observed distribution with expectation follows:

		Normal	*Zigzag*	*Total*
Group 1				
	Observed	2153	138	2291
	Expected (15 : 1)	*2148*	*143*	*2291*
	Deviation, 5.0 ± 24.7			
Group 2		*Normal*	*Zigzag*	*Total*
	Observed	759	262	1021
	Expected (3 : 1)	*766*	*255*	*1021*
	Deviation, 7.0 ± 9.3			

For each group the deviation from expectation is less than the probable error. Deviations as large as these are to be expected from chance alone in more than one out of every two similar trials.

F_1 plants back-crossed with zigzag plants

Two F_1 plants from the cross, normal × zigzag, were back-crossed with zigzag plants. The same F_1 plants were self-pollinated and gave 15 : 1 ratios in the F_2 generation, thereby indicating that both factors for zigzag culms were involved. The progenies obtained from the back-crosses consisted of normal and zigzag plants in ratios approaching the 3 : 1 relation expected. The numbers observed are given in table 2.

TABLE 2

F_1 progenies of the cross $Z_g z_g Z z \times z_g z_g z z$.

PEDIGREE NUMBERS	NORMAL CULMS	ZIGZAG CULMS	RATIO
255	251	75	3.35 : 1
256	163	56	2.91 : 1
Total	414	131	3.16 : 1
Expected	*408.75*	*136.25*	*3.00 : 1*

Deviation, 5.25 ± 6.8

Further tests of the back-crossed plants

To further substantiate the conclusions drawn, many normal plants from two dihybrid back-cross cultures were self-pollinated or again back-crossed with zigzag plants. The results are given in table 3.

In many of the progenies the number of plants is small and in some the deviation from the expected ratio is quite large. But the dihybrid and monohybrid progenies are sharply separated. The totals for both families are given in table 4.

The expected proportions of genotypes in a dihybrid back-cross population are as follows:

1 $Z_g z_g Z z$, normal dihybrid
1 $Z_g z_g z z$, normal monohybrid
1 $z_g z_g Z z$, normal monohybrid
1 $z_g z_g z z$, zigzag

One-third of the normal plants should give dihybrid progenies when self-pollinated or crossed with zigzag and two-thirds should give monohybrid progenies. Thirty-four normal plants of family 255 were tested and it was found that 12 were dihybrid and 22 monohybrid. Also 28 normal

TABLE 3

Progenies of the back-cross normal plants when self-pollinated or again crossed with zigzag.

PLANT NUMBER	SELF-POLLINATED			CROSSED WITH ZIGZAG PLANTS		
	Normal	Zigzag	Ratio	Normal	Zigzag	Ratio
Family 255						
Dihybrid						
5	61	2	30.5 : 1	15	4	3.75 : 1
6	62	6	10.3 : 1	59	24	2.71 : 1
8	65	5	13.0 : 1			
10				72	23	3.13 : 1
18	83	6	14.0 : 1	58	18	3.22 : 1
27	83	6	14.0 : 1			
29				57	16	3.56 : 1
31	65	5	13.0 : 1	70	24	2.92 : 1
38	71	3	23.6 : 1			
45	69	4	17.2 : 1			
48				58	16	3.62 : 1
64	80	5	16.0 : 1	51	23	2.22 : 1
Total	639	42	15.2 : 1	440	148	2.97 : 1
Expected	*638.5*	*42.5*	*15.0 : 1*	*441*	*147*	*3.00 : 1*
Monohybrid						
7				37	37	1.00 : 1
11				23	24	0.96 : 1
12	56	15	3.73 : 1			
13	58	19	3.05 : 1			
16	67	28	2.40 : 1	19	21	0.90 : 1
20	32	12	2.67 : 1			
21	50	23	2.17 : 1			
22	56	16	3.50 : 1			
24	64	18	3.56 : 1			
25	62	20	3.10 : 1			
28	62	21	2.95 : 1			
30	48	20	2.40 : 1			
32	48	15	3.20 : 1	38	35	1.09 : 1
35	54	21	2.57 : 1			
39	61	20	3.05 : 1			
44	49	25	1.96 : 1			
47	65	22	2.95 : 1			
49	49	15	3.27 : 1			
51	68	25	2.75 : 1			
54	57	20	2.85 : 1			
57	50	16	3.12 : 1	13	4	3.25 : 1
61	56	17	3.30 : 1			
Total	1112	388	2.87 : 1	130	121	1.07 : 1
Expected	*1125*	*375*	*3.00 : 1*	*125.5*	*125.5*	*1.00 : 1*

TABLE 3 (continued)

PLANT NUMBER	SELF-POLLINATED			CROSSED WITH ZIGZAG PLANTS		
	Normal	Zigzag	Ratio	Normal	Zigzag	Ratio
Family 256 Dihybrid						
23	106	6	17.7 : 1			
26				54	12	4.50 : 1
46	84	8	10.5 : 1			
48				26	9	2.89 : 1
52	47	1	47.0 : 1	27	2	13.50 : 1
56	61	1	61.0 : 1			
57	96	6	16.0 : 1			
58				71	10	7.10 : 1
60				40	15	2.67 : 1
64	92	2	46.0 : 1			
66	57	6	9.5 : 1	52	20	2.60 : 1
67	46	3	15.3 : 1			
Total	589	33	17.85 : 1	270	68	4.00 : 1
Expected	*583*	*39*	*15.00 : 1*	*253.5*	*84.5*	*3.00 : 1*
Monohybrids						
1	137	26	5.27 : 1			
2	53	16	3.31 : 1			
4	43	15	2.87 : 1	4	1	4.00 : 1
5	65	20	3.25 : 1			
6	77	20	3.85 : 1			
8	51	17	3.00 : 1			
18	39	17	2.30 : 1			
24				44	28	1.57 : 1
27	38	34	1.12 : 1			
32	49	14	3.50 : 1			
39				22	25	0.88 : 1
40				39	26	1.50 : 1
43	28	13	2.15 : 1	41	25	1.64 : 1
50	49	29	1.70 : 1	33	19	1.74 : 1
53	56	17	3.30 : 1			
59				11	5	2.20 : 1
Total	685	238	2.88 : 1	194	129	1.50 : 1
Expected	*692.25*	*230.75*	*3.00 : 1*	*161.5*	*161.5*	*1.00 : 1*

TABLE 4

	SELF-POLLINATED				CROSSED WITH ZIGZAG			
	Normal	Zigzag	Total	Ratio	Normal	Zigzag	Total	Ratio
Dihybrid progenies								
Observed........	1228	75	1303	16.4 : 1	710	216	926	3.28 : 1
Expected.........	*1221.5*	*81.5*	*1303*	*15 : 1*	*694.5*	*231.5*	*926*	*3.00 : 1*
Deviation........	6.5 ± 18.6				15.5 ± 8.9			
Monohybrid progenies								
Observed........	1797	626	2424	2.87 : 1	324	250	574	1.3 : 1
Expected.........	*1817*	*606*	*2423*	*3 : 1*	*287*	*287*	*574*	*1.0 : 1*
Deviation........	20.0 ± 14.4				37.0 + 8.1			

plants of family 256 were tested and it was found that 12 of them were dihybrid and 16 monohybrid. The totals taken from table 3 are as follows:

	Dihybrid	*Monohybrid*	*Ratio*
Observed.	24	38	1 : 1.58
Expected..	*20.7*	*41.4*	*1 : 2 00*

Deviation, 3.3 ± 2.5

A deviation as large as this could be expected about once in every three trials, due to chance.

LINKAGE BETWEEN THE FACTOR PAIRS $Z_g\ z_g$ AND $G_s\ g_s$

Plants with zigzag culms were crossed with green-striped plants (LINDSTROM 1918). The F_1 plants were normal both for culm form and for leaf color. Two F_2 distributions involving one of the zigzag factors and the factor for green striping are given in table 5. No green-striped zigzag plants were obtained. The other classes were as follows: 378 plants with green leaves and normal culms ($G_s\ Z_g$), 200 plants with green leaves and zigzag culms ($G_s\ z_g$), and 203 plants with green-striped leaves and normal culms ($g_s\ Z_g$). The absence of green-striped zigzag plants, together with the close approximation to a 2 : 1 : 1 : 0 ratio, indicates close or complete linkage between the factor pair G_sg_s and one of the factor pairs for zigzag culms. To this zigzag factor pair the symbols Z_gz_g have been assigned.

TABLE 5

F_2 progenies of the cross $z_g\,z_g\,z\,z\,G_s\,G_s \times Z_g\,Z_g\,z\,z\,g_s\,g_s$.

PEDIGREE NUMBERS	GREEN PLANTS		GREEN STRIPED PLANTS	
	Normal	Zigzag	Normal	Zigzag
1527	193	93	98	0
1528	185	107	105	0
Total	378	200	203	0
Expected (complete linkage between z_g and g_s)	*390.5*	*195.25*	*195.25*	*0*
Deviations..............	−12.5	4.75	7.75	0

$\chi^2 = 0.824$ P = slightly less than 1.

Table 5 gives a summary of data on F_2 distributions involving green-striped leaves and both factors for zigzag culms. The results are in agreement with the expected ratio (11 : 1 : 4 : 0) for complete linkage between the factor pairs $Z_g\,z_g$ and $G_s\,g_s$.

TABLE 6

F_2 progeny of the cross $z_g\,z_g\,z\,z\,G_s\,G_s \times Z_g\,Z_g\,Z\,Z\,g_s\,g_s$. Family 1525.

	GREEN PLANTS		GREEN STRIPED PLANTS	
	Normal	Zigzag	Normal	Zigzag
Observed................	241	19	82	0
Expected (11 : 1 : 4 : 0)....	*235.1*	*21.4*	*85.5*	*0*
Deviations..............	5.9	−2.4	−3.5	0

$\chi^2 = 0.561$ P = slightly less than 1.

The value of χ^2 for the data in table 5 is 0.824, and for the data in table 6 χ^2 is 0.561. Thus it is seen that in each case the observed numbers are in close agreement with expectation.

SUMMARY

Maize plants with zigzag culms were first observed in a number of F_4 progenies of a cross between Tom Thumb pop corn and a variety of Missouri dent corn. The culms are more or less crooked instead of straight as in normal plants. The affected internodes arch away from the point of leaf attachment, and in many instances the internodes are entirely removed from the leaf sheaths which normally clasp them.

Crosses between normal plants and zigzag plants give F_1 plants with normal culms. Some F_2 progenies consist of normal plants and zigzag plants

in the ratio 15 : 1; others have these phenotypes in the ratio 3 : 1. The data are explained by assuming two independent factors. These factors have been designated Z_g z_g and Z z. Zigzag culms appear only when both factor pairs are recessive.

The factor pair Z_g z_g is very closely or completely linked with the factor pair G_s g_s.

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A RARE CARBOHYDRATE IN WAXY MAIZE

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The dextrins, which occur as successive steps in the transition from glucose to starch and from starch to glucose, are so rarely detected in plant tissues and so ephemeral in existence, that their occurrence in stable form in matured or dormant tissues has seldom been reported. It has been the occasion of no little interest, therefore, to find that the sole carbohydrate in the endosperm of "waxy" maize is the rare erythrodextrin.[1]

This variety of corn has been known to science since 1909, when COLLINS (1909) described it as a new type found in China. Besides possessing certain vegetative peculiarities, it has an endosperm distinctly different from that of other varieties, justifying its recognition as a cultural variety coördinate with the flints, dents, pops, etc.

The grains of waxy corn are rounded and plump, showing no roughened exterior due to shrinkage at maturity. The endosperm, although hard like that of flint corn, is opaque, and shows a dull, smooth fracture.

Since its discovery in China, this variety has also been found in Burma (COLLINS 1920), and its occurrence in these two places has been considered in connection with claims of a pre-Columbian occurrence of maize in the Orient. The peculiar type of endosperm has also been extensively studied genetically. No one, however, seems to have explained, thus far, the chemical and physical basis of the waxy character itself. COLLINS (1909, p. 15) states: "The appearance and physical composition of the seeds of this Chinese corn were so distinct from that of other varieties that the possibility of a difference in chemical composition naturally suggested itself, but analyses did not yield any very unusual results." Then follow data from analyses made by the Bureau of Chemistry, of twenty-one varieties of corn, including the new Chinese type and a hybrid between it and a corneous variety. Inasmuch as appreciable oil contents are noted, it seems that the whole grains were analyzed, and such an analysis could not be expected to indicate in any accurate way the composition of the endosperm, the structure under investigation.

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[1] A dextrin which is turned red by iodine. Amylodextrin turns blue, and achroödextrin gives no color reaction with iodine. The term "amylodextrin" is sometimes used for all dextrins regardless of their color reactions with iodine. Here and elsewhere in this paper, the iodine reagent to which reference is made is an aqueous solution, of various strengths, of one part of iodine and four parts of potassium iodide.

The nature and quantity of carbohydrate present appear to have been ignored in these analyses. In his extended report on the inheritance of the waxy endosperm, KEMPTON (1919) makes no mention of any further attempts to determine the basis of the peculiarity.

The peculiar texture of the endosperm of waxy maize seems to be determined wholly by the physical properties of the grains of carbohydrate in its cells. The iodine test for starch in this endosperm brings out a rich violet-red color such as is given by no carbohydrate other than erythrodextrin. On microscopic examination the dextrin is found to be in the form of definite grains similar in size and shape to the starch grains of other varieties of maize, but less transparent. This dextrin is soluble in hot water, the solution or viscous suspension giving the characteristic red reaction with iodine. It dissolves very slowly in cold water, probably because of its granular form. It is readily hydrolyzed by diastase.

The only comprehensive account that has been found of this polysaccharide is that by ARTHUR MEYER (1886). From the data accumulated from his own researches and the works of NÄGELI, GRIS, SACHS, TREUB, RÜSSOW, and DAFERT, he finds that starch grains which are turned red by iodine are known to occur in *Iris germanica*, *Chelidonium majus*, *Acer Pseudoplatanus*, *Monotropa Hypopytis*, *Gentiana lutea*, *Swertia perennis*, eight species of orchids, and three cereals. The latter, *Oryza sativa glutinosa*, *Panicum miliaceum glutinosum*, and *Sorghum vulgare glutinosum*, are of special interest here because of their relationship to maize. PARNELL (1921) states that a dextrin of this kind is found in mace and a small group of varieties of rice. This must also be the carbohydrate mentioned by YAMAGUCHI (1918) n a variety of rice which shows xenia when pollenized by other varieties.

In the cases mentioned by MEYER (1886), the dextrin is found sometimes in the seeds and sometimes in vegetative parts of the plants mentioned, its grains often being associated with those of ordinary starch. In the glutinous variety of sorghum it occurs only in the endosperm, the starch of the embryo and other vegetative parts showing the blue reaction with iodine. PARNELL (1921) reports a distinct difference in reaction between the pollen grains of glutinous and starchy varieties of rice.

In waxy maize the erythrodextrin has been found only in the endosperm. The carbohydrate in the embryo is ordinary starch. That in other parts of the plant has not been examined.

The occurrence of erythrodextrin in maize has a significance other than that of a mere addition to a meagre list of plants showing the characteristic. The difference in genetic behavior between waxy corn and sweet corn has a close parallel in the microchemical differences between the two; and the peculiar chemical condition here described for the one may throw into greater relief some of the significant facts about the endosperm of the other.

Geneticists of the past quarter of a century have found in hybrids between sweet and starchy varieties of maize, material readily utilizable for illustrating Mendelian ratios. But in the majority of the experiments reported, the 3 to 1 ratio expected in the conventional F_2 generation (of endosperms) has been complicated by the occurrence of grains intermediate between sweet and starchy. The usual recourse has been the citation of the possibility of a reclassification, based upon the degree of expression of the characters concerned, or an interpretation based upon various observed or assumed conditions. On the other hand, the waxy character of the endosperm behaves as a simple recessive to the corneous condition, the two characters being definitely alternative, "no blending or intermediate stages having been found" (KEMPTON 1919).

The endosperm of sweet corn seems to contain many hexose compounds. The most prominent objects encountered in a microchemical examination are starch grains with rough, eroded surfaces, suggesting partial hydrolysis during maturity of the endosperm. These grade off through a series of smaller grains to an amorphous mass, which becomes glutinous when soaked in water. These carbohydrates are probably starch and amylodextrin, since they give the blue reaction with iodine. The sweet taste, more or less of which remains in the mature endosperm, suggests the presence of achroödextrin, reducing sugars, or sucrose. Mature seeds, however, usually give negative results when tested for reducing sugars. An occasional violet reaction with iodine indicates the presence of some erythrodextrin, but definite grains having this reaction have not been found.

Since the enzymes concerned in the synthesis of the reserve materials in such tissues as this are known to be often reversible with temperature changes, it seems probable that the peculiar organization of the carbohydrates in sweet corn may be due to repeated reversals of the process during the maturity of the seed. This is further indicated by the fluctuating sweetness of sweet corn in the edible stage, depending upon the time of harvesting and conditions of storage.

In contrast with this condition, the endosperm of waxy corn contains but the one kind of carbohydrate, and its grains show no indication of partial hydrolysis as do those of sweet corn.

Sufficient consideration of the fundamental physical and chemical nature of the endosperm of sweet corn in connection with experimental work in the past few years would have done much to clear up a muddled situation by affording a more definite basis for the classification of intermediate grains and of others which have given unexpected genetic results. Much fruitless supplementary experimental work undertaken in attempts to explain unexpected results could have been avoided if the investigator had known definitely with what characters he was dealing, and a better knowledge of the real nature of the characters concerned will doubtless show that such widely divergent views as those of HARPER (1920) on the one hand and the main body of geneticists on the other, are really not so far apart. This clearer understanding would have enabled COLLINS and KEMPTON (1914) to determine the real nature of the grains that resembled starchy corn in contour and sweet corn in heredity, and would have pointed the way toward more conclusive results in JONES's (1919) work on "pseudo-starchy" endosperm.

The smooth contour of an apparently starchy grain of corn may cover a multitude of possibilities, and the visual method of discriminating between endosperm textures by observing the outside of the grain is as crude as a color-blind man's attempt to describe the rainbow. That intermediates between the two widely different types of endosperm (sweet and starchy) do occur is not to be doubted, and many grains apparently sweet or starchy will doubtless fall into intermediate classes as our vision becomes keener. From what we know at present, nothing less than the assumption of many factorial differences between sweet corn and starchy corn will be in accord with our genetic and histological data, and even then a generous allowance must be made for the effect of environment.

Of geographical interest, although without any significance known at present, is the generalization that all four of these cereals,—rice, sorghum, millet, and maize,—known to have erythrodextrin in their endosperms, have been first found in eastern Asia and the adjoining islands.

Waxy maize has doubtless arisen from starchy maize by mutation, and the nature of its endosperm indicates that its origin has been as simple as that of sweet corn, since only the size of the molecule of reserve material has been affected. Inasmuch as the sporadic appearance of sweet corn as a mutant from starchy corn in many parts of America, is known, the occurrence of waxy maize in China and in upper Burma is probably of no great significance as an indication of the pre-Columbian occurrence of maize in Asia.

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HEREDITY OF ANGIONEUROTIC EDEMA BASED ON A REVIEW OF THE LITERATURE

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Angioneurotic edema is manifested clinically by sudden swellings of the skin or mucous membranes, which persist for a few hours to several days, and disappear as quickly as they come. There may be only a single outbreak during the life of an individual or the swellings may occur at irregular intervals with greater or less frequency, so that in some cases the life of the victim may become burdensome. In some cases the attacks are periodic. The size of the swellings varies, at times being enormous. In certain positions they may cause death by mechanical interference with one of the vital functions. This is most common in edema of the glottis.

The disease may appear in childhood or it may not be noticed until past middle age. Usually it becomes less annoying as old age approaches.

In the past the causes of the disease were given by various authors as "nervous influences," "infections," "disorders of the endocrine glands," and "dietetic errors." At present allergy would seem to be the most plausible explanation and this is substantiated in certain types of the disease. Whether allergic individuals are born hypersensitive to specific substances, or whether they are born with a tendency to become easily sensitized is still a much disputed subject. COCA (1920) is quite positive that, unlike experimental anaphylaxis, it is not an antibody-antigen reaction. On the other hand, SCHLOSS (1920) reports the passive transference of sensitization from children to guinea-pigs by means of the blood serum.

However this may be, it is known that in some cases the ingestion of certain foods causes the attack and that when these foods are not eaten, the attacks do not occur, and that in many of these, but not all, a positive skin test to the protein of the offending food can be demonstrated. In some cases where the skin tests are positive to specific foods, abstinence from them does not always prevent the attacks. In others the disease may be shown clinically to depend on a food and the skin test to it may be negative even though its removal from the dietary apparently prevents the outbreaks.

From a clinical standpoint these definitely allergic cases may be roughly grouped into two types which we will designate as the prompt and the delayed.

In the prompt type within a comparatively short time after the offending protein is taken, nausea, vomiting, and perhaps diarrhoea, appear, and these are quickly followed by areas of pruritis and wheals which rapidly coalesce. The edema soon assumes remarkable proportions especially about the head and neck. Skin tests are often positive. Adrenalin may be potent to control the attack. In many individuals some of the outbreaks never go beyond the urticarial stage, while others become edematous.

This type of angioneurotic edema closely resembles certain cases of general allergic reaction which are produced in hypersensitive individuals when they are injected with the various substances to which they react. (WALKER 1918; COOKE 1922.)

In the delayed type some hours or even days may elapse after the ingestion of the offending food before symptoms appear. The lesions are usually single and commence with a slight tingling or pricking sensation, and expand with remarkable speed until they become superwheals. Gastro-intestinal crises may or may not be present. Adrenalin, even when injected into the swellings, is apparently valueless in controlling them. As a rule, skin tests are negative, but there are exceptions, so that the determination of the offending substance rests upon a clinical basis only. For this reason the cause of the disturbance is not ascertained in many cases which are definitely hypersensitive; consequently, their treatment is unsatisfactory and difficult.

OSLER (1897) suggests that changes occurring in the food during some stage of its sojourn in the body may be responsible for some of these cases.

Since the time of QUINCKE (1882) angioneurotic edema has been recognized as heritable, and indeed many authors state that it seems to be a Mendelian dominant, but we know of no one who has made an analysis of the reported families. From a genetic standpoint the disease can be classified as sporadic and familial.

SPORADIC ANGIONEUROTIC EDEMA

The so-called sporadic cases deserve minute investigation. Allergy is certainly the fundamental condition in most of them, and the family history often reveals the presence of eczema, urticaria, hay-fever, asthma, etc. An example of this is shown in figure 1 (PHILLIPS 1922). Indeed, its association with these diseases, which also depend upon allergy, is so common as to suggest the possibility that the heredity of these diseases depends upon more than one factor.

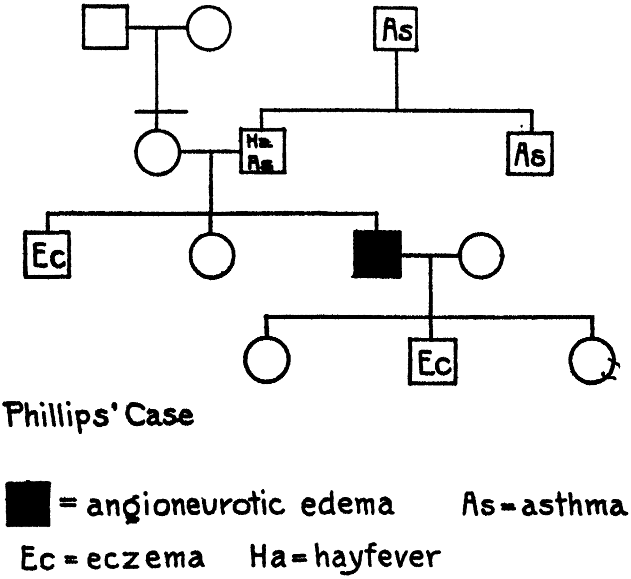

FIGURE 1.—From PHILLIPS (1922), illustrating a sporadic case in which eczema and asthma are familial traits. Note that in this family all males are affected with some manifestation of sensitization.

It may be noted here that the visible lesions of allergy are those of hyperemia and edema. Thus in the skin test (BROWN 1922) a positive result is shown by erythema followed by the formation of a wheal, with irregular outlines and pseudopod-like extensions of the edema, which apparently follow the course of the lymphatics. In the erythemas the hyperemia is more noticeable than the edema. These conditions are reversed in urticaria. Hay-fever is characterized by the development of a similar hyperemia and edema of the mucous membranes of the eye, nose and throat. However, the appearance of this edema is somewhat modified by a secondary bacterial infection of the swollen mucous membrane. Certain types of eczema would seem to show a like series of changes occurring in the skin. These are further complicated by trauma, inflicted by the patient as he scratches his itching skin, and by secondary infection.

Most authors consider asthma due to a spasm of the non-striated muscle of the bronchi, but the appearance of the mucous-membrane lining of the trachea and bronchi, as described by FREUDENTHAL (1912) who viewed them through the bronchoscope during attacks, would seem to show definitely that here again we have erythema and edema of the mucous membrane.

The rheumatoid pains so often associated with urticaria and angioneurotic edema, are probably due to a similar condition of the serous membrane of the joints.

In angioneurotic edema this edema seems to be more generalized and intense, so that the skin, mucous, and serous membranes, and their underlying connective tissues, are all affected.

As all of these diseases are well known to be heritable, and are often associated as family traits, it would seem that the main difference between them lies in the distribution of hypersensitive tissues and in the intensity of their reaction. The cause of these patterns of distribution of hypersusceptibility of the tissues and the variation in its intensity should be investigated by the geneticist.

The solution is rendered difficult not only because the hypersensitiveness is not specific, as various members of the same family respond to different proteins, but also on account of the varying age at which symptoms appear. COOKE and VANDERVEER (1917) have called attention to the fact that symptoms occur earliest in those individuals who inherit this tendency from both parents. They consider allergy as a dominant trait while ADKINSON (1920) investigating the heredity of asthma as a specific disease, concluded that it was a recessive.

Another of the many difficulties is that a person may be hypersensitive, but, unless the environment is such that he comes in contact with his allergic substance, he will remain ignorant of his condition. Thus we know of one family of Germans in which ragweed hay-fever appeared for the first time in many of its members the very first fall after immigrating to this country, those afflicted ranging in age from the grandfather to a young child. Such persons might easily appear in a pedigree as normals.

Despite all these obstacles we believe that full pedigrees of victims of angioneurotic edema of the sporadic type from hypersensitive families, including all normal members as well as those afflicted with the various clinical types of allergy, might aid much toward unraveling this mystery.

FAMILIAL ANGIONEUROTIC EDEMA

In the familial types the attacks appear generation after generation in many members of the afflicted families and exact a heavy toll of suffocative deaths caused by edema of the glottis. These characteristics are shown in figure 2. The symptoms are so like those occurring in the sporadic type that it is generally considered to be an identical condition, but we are not aware of the skin reactions of any of these families having been made the subject of research by the immunologist. Studies of the protein reactions in one of these families, together with a complete family history covering all diseases of allergic origin, might do much toward solving the problem of the heredity of human allergy.

A careful study by matings of the twenty-seven pedigrees published in the Treasury of Human Inheritance (Bullock 1909), which appears to bring the data up to its date, together with the pedigrees of Bolten (1919), Cameron (1920), Crowder and Crowder (1917), and Edgerly and Lusk (1919), which have been published later, has been summarized in tables 1 and 2.

TABLE 1

Affected × unaffected.

CHARACTERISTICS OF PROGENY	MALE PARENT AFFECTED. 56 MATINGS			FEMALE PARENT AFFECTED. 46 MATINGS			SUMMARY 102 MATINGS			
	Offspring	Percent		Offspring	Percent		Sum	Sex?	Sum	Percent
Affected males	70	35	53.1	29	27.6	44.9*	99	3	168	50.15
				3†						
Affected females	45	22.5		21	20		66			
Unaffected males	47	23.5	47.1*	27	25.7	55.1*	74	27	167	49.85
	17†			10†						
Unaffected females	38	19		28	26.7		66			

*Including those of unknown sex.
†Sex unknown.

From tables 1 and 2 it will be seen that only two types of matings occur in the pedigrees, namely, matings of affected individuals with unaffected and matings of unaffected with unaffected. Taking up the matings of affected × unaffected first (table 1), the figures show conclusively that familial angioneurotic edema may be transmitted by either parent equally well, the disease appearing in approximately half of the offspring. When the male parent is the one affected the proportions of

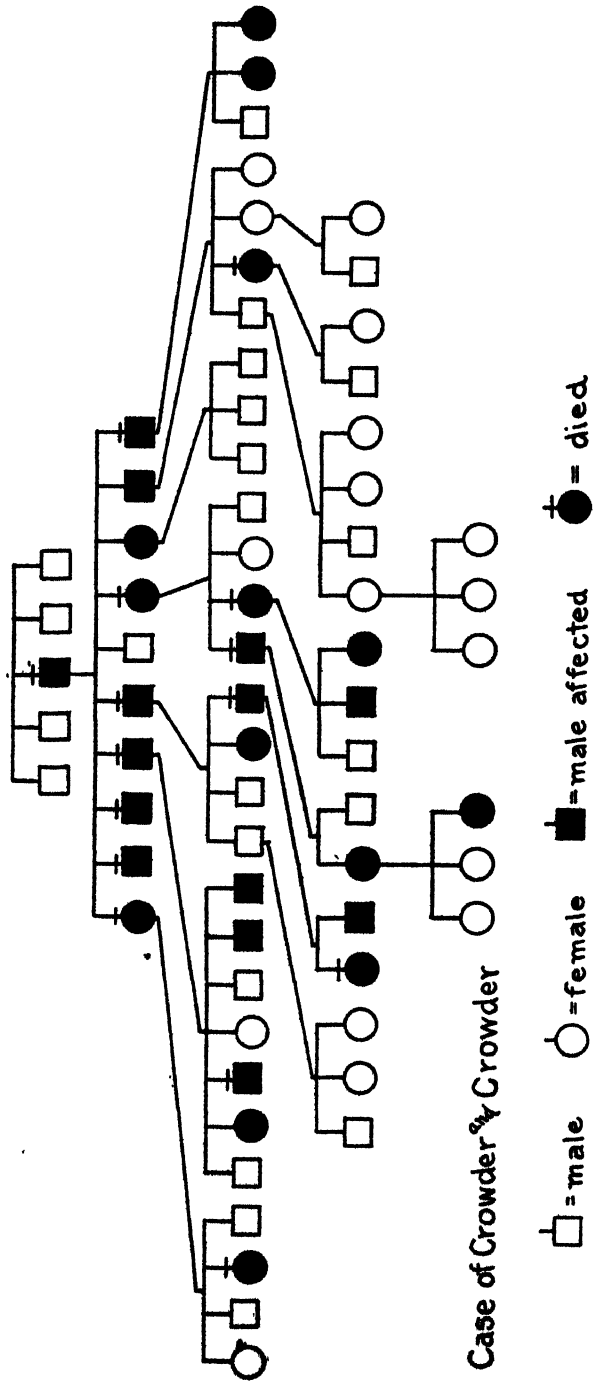

FIGURE 2.—FROM CROWDER and CROWDER (1917), illustrating a typical familial angioneurotic edema. Note the high mortality due to glottis edema.

TABLE 2

Neither parent affected.

CHARACTERISTICS OF PROGENY	PRODUCING NO AFFECTED PROGENY	PRODUCING AFFECTED PROGENY	
	One grandparent* affected 27 matings	One grandparent affected 10 matings	Grandparents unknown 6 matings
Affected males	0	12	6
Affected females	0	5	3
Unaffected males	32	6	2
Unaffected females	49	7	3

*In one mating the great-grandparent only was affected.

affected are larger than would be expected. This difference is in the opposite direction when the female is the affected parent. Affected males are more numerous than affected females. This is what one would expect since males are more numerous in the whole population. However, the excess of affected males over unaffected males (99–74 = 25) is considerable when compared with the females among which the affected and unaffected are equal in numbers. Many authors still consider human sensitization as an expression of anaphylaxis. If familial angioneurotic edema proves

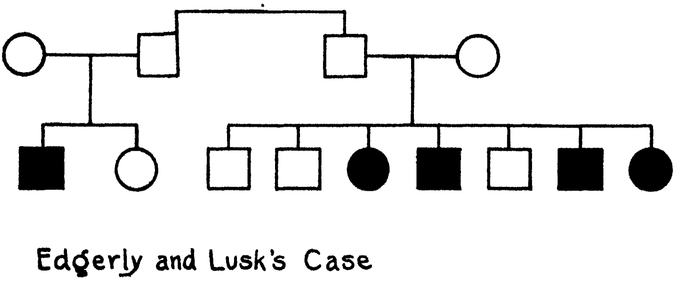

FIGURE 3.—From EDGERLY and LUSK (1919), illustrating two unaffected brothers mated to unaffected females, each producing affected offspring.

to be due to allergy, this type of inheritance forms a striking contrast to the so-called inheritance of laboratory anaphylaxis in which the offspring of the females only are affected because the sensitization is due to a passive transfer of antibodies. If table 1 were our sole source of material we would say without hesitation that angioneurotic edema is a dominant trait and that all matings recorded in the table were of the DR × RR type, giving DR and RR offspring in equal numbers. The matings recorded in table 2 throw considerable doubt on this interpretation.

For the sake of ease in analysis table 2 is divided into two parts according to the progeny. The second of these groups is again divided on the basis of whether the grandparents were affected or were unknown. The second category probably belongs with the first, but since nothing is known about the grandparents it is of little value. The results recorded in the first column of the table show that we are here dealing with parents which are recessives, i. e., normals, the matings being of the type RR × RR giving RR offspring only. This is what would be expected if the trait were a dominant. Each of the ten matings recorded in the next column produced affected individuals. These with the six matings of the next column indicate that in sixteen out of a total of forty-three matings, or 37 percent, the parents carried the trait without showing it. These figures are too large to be due entirely to accidental errors.

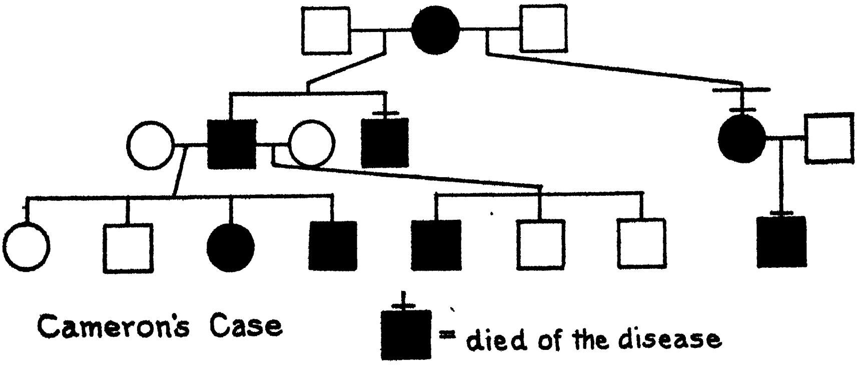

Figure 4.—From Cameron (1920), illustrating the typical dominant type of inheritance through two double matings.

Two other explanations are possible; firstly, that angioneurotic edema is due to a fundamental condition which requires some specific stimulus to disclose it and that these cases were potentially affected but were not exposed to the proper stimulus to make the disease appear; or secondly, that the condition is due to two factors which are closely linked. If the second explanation is the correct one, each of two unaffected parents supply one of these factors, which after crossing over are transmitted together more often than they are separated. This would account for their inheritance in most cases as a dominant trait. It would also account for the occasional production of an affected individual from two unaffected parents each alone incapable of producing edema. One of these factors may very likely be the factor which makes the bearer respond to some exciting substance by developing asthma, eczema, hay-fever, or urticaria. The other factor, if such is present, is either similar to the first or unknown. That urticaria, asthma, eczema, hay-fever and other allergic conditions are very common in families where angioneurotic edema occurs has long been known. Sometimes angioneurotic edema and asthma are found together in the same person as in Bolten's (1919) case, figure 5. In this case the patient had angioneurotic edema, urticaria, perennial hay-fever and tetany, while a cousin had angioneurotic edema and asthma.

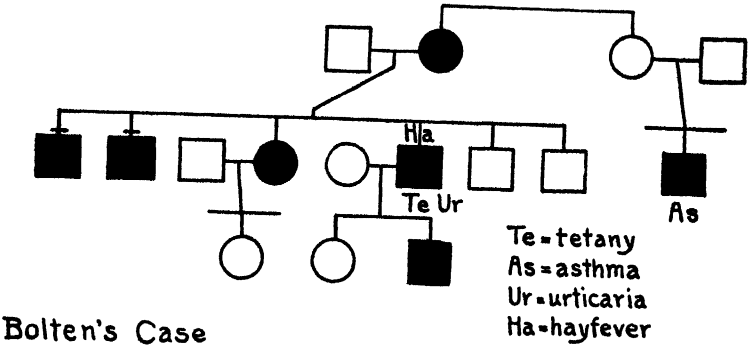

Figure 5.—From Bolten (1919), illustrating familial angioneurotic edema associated with other manifestations of allergy.

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HOLOGYNIC HEREDITY

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Sex-linked characters in man are well known; they are all pathological characters, so far as known; they are transmitted according to the same laws as the sex-linked characters in the little fruit-fly (Drosophila) and explained in the same manner by means of the sex chromosome.

I propose the name "diagynic heredity" for this kind of heredity, as the sex-linked character is transmitted from male to male, not directly, but through the unaffected females (e. g., hemophilia, daltonism).

In the currant moth (*Abraxas grossulariata*) the character "*lacticolor*" is transmitted from female to female, not directly, but through the "unaffected" males. This "dia-andric heredity" is not known in man.

A third kind of sex-linked heredity is possible. We know two pedigrees of this kind (daltonism, hemophilia), and two new pedigrees are referred to in this paper (hemophilia, cataract). No one has considered this special kind of heredity or noticed its theoretical and practical importance.

For the pedigree of daltonism (CUNIER), see PLATE 1913, p. 379. Daltonism is usually diagynic; but in this pedigree 12 females are affected in 5 generations. The authors have not emphasized the circumstance that *all* the females are affected. The males are all unaffected. The transmission is direct from the affected female to her daughters.

In another pedigree of hemophilia (GRANDIDIER, see PLATE 1913, p. 379), an affected mother has three affected daughters and no other children; her parents, according to the reference, are unaffected.

I propose for this type of heredity the name of "hologynic heredity."

"Holo-andric heredity" has not been recognized, heretofore, but in the *Journal of Heredity* for November 1921 RICHARD SCHOFIELD has published a pedigree of webbed toes with 14 affected males in 4 generations (all the males) and not one female. SCHOFIELD does not recognize in this pedigree a new form of heredity and thinks that this hereditary trait is inherited as a secondary sex character. But why are *all* the males affected?

This is probably the first known case of holandric heredity.

Two other pedigrees of hologynic heredity will be presented here. The first is very small and concerns again hemophilia (figure 1). In the third generation the first daughter is probably becoming affected, but this is not yet certain.

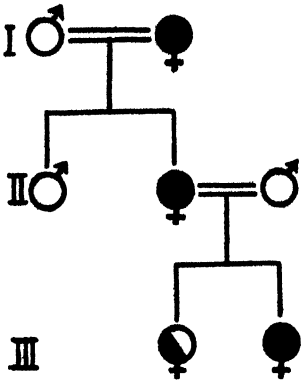

FIGURE 1.—Pedigree chart of a family showing hemophilia in the female line,—hologynic hemophilia.

Much more interesting is the second pedigree, of senile cataract (figure 2). My friend, Prof. GILBERTO ROSSI, the well known physiologist in Florence, has had the kindness to compile this pedigree and permit me to publish it. I am much indebted to him for this.

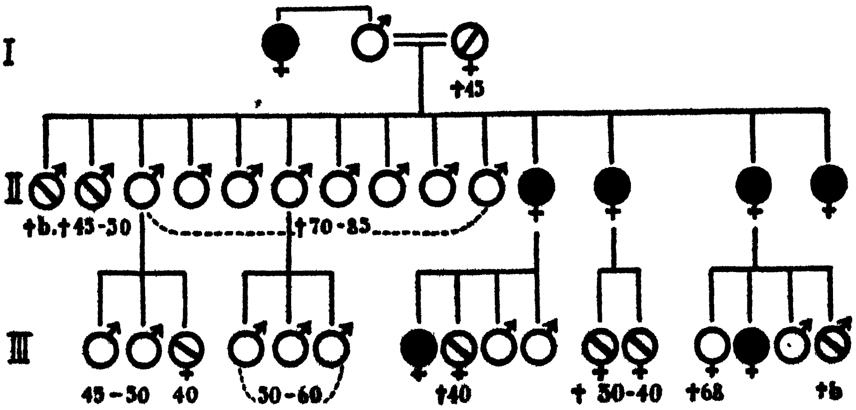

FIGURE 2.—Hologynic senile cataract. The numbers indicate the age that the individual, if living, now has, or the age at death. An oblique line from left to right indicates that the individual has not reached the age in which the cataract developed; from right to left that no information is available as to whether the individual was affected or not; b indicates that the individual died as a child.

In the family of this pedigree, as the females approach the age of 50 years they expect to become affected with cataract. Really only one female has had the good luck to constitute an exception to this rule; she has reached 68 years with good sight. Fourteen males have arrived at or surpassed 50 years, but not one of them has become affected.

The pedigree begins with a couple of which little information about the female is available, except that she died at an age (45) at which the affection is not noted at all in the other females of this family. The sister of her husband was affected.

We cannot determine from this pedigree, or from the others of hologynic heredity, whether the males can be conductors; it is not known whether the cataract is brought into the family by the male of the first generation as a conductor, or by the female.

Notwithstanding the fact that this pedigree is not so extensive as to solve all the questions which suggest themselves, it is clear that we have before us here a new form of heredity.

The direct transmission from female to female and the affection of *all* the females exclude it as a case of dia-andric heredity; the number of the affected females and of the unaffected males exclude also the hypothesis that the affection might be dominant and independent of sex.

The same conclusion is justified in the case of hemophilia. This affection is usually diagynic and females are usually unaffected, for affected females could be born only if the father is affected and the mother is a conductress. But DAVENPORT (1911) has published a pedigree of hemophilia with some affected females. He noted this abnormality, but did not point out that the method of transmission is in this case very different from the usual diagynic; the hemophilia is here dominant and independent of sex. There are two other unusual pedigrees of hemophilia, one of GRANDIDIER, and the one referred to in this paper (figure 1), in which only the females are affected.

Daltonism is usually inherited as a diagynic trait; but an unusual pedigree is known with affected females, and in this case all the females (12) are affected, with direct transmission, and not one male. It is not admissible to assume that in all these cases the affection is a dominant one, independent of sex, and that by chance only females (all the females) are affected. Such a possibility exists, to be sure; if we find a short pedigree of this kind among ten thousand ordinary pedigrees of brachydactyly, for instance, this may be by chance. But we know only three extraordinary pedigrees of hemophilia, with many affected females, and in two of these all the females are affected; of two cases of daltonism one is also of this kind. The pedigree of cataract shown in figure 2, also can not be reasonably explained on the ground of chance.

Thus, a new kind of heredity exists, sex-linked, but not diagynic or dia-andric,—hologynic heredity. The features of this heredity are not yet known, but its existence seems to me to be certain. Provisionally I suppose that these features are very simple: (1) The daughters of an affected mother are all affected; (2) The sons of an affected mother are all unaffected and not conductors.

How can hologynic heredity be explained? The coexistence of diagynic and hologynic heredity in the same species makes the explanation very difficult. Nevertheless, I will make an attempt in this direction.

The diagynic heredity requires the existence of two kinds of spermatozoa, one of which possesses the X chromosome, the other not. Naturally, it is possible that paired with the X chromosome there is a Y chromosome, different from X, and destined to remain always in the male line. That is really the case in some kinds of insects. If one should discover a holandric heredity in the insects, its explanation would be very easy; the Y chromosome, which remains in the male line, could well explain a sex-linked character which remains always in the male line.

But we have to explain a hologynic, not a holandric, heredity. We can imagine a scheme like that for the dia-andric heredity, with two kinds of eggs and a W chromosome which remains always in the female line. W can transmit the cataract or the hemophilia or the daltonism.

This explanation or a similar one is logically suggested by the observed facts. It is necessary to suppose that something remains in the female line and can not be transmitted by the male. Then an egg possesses this thing or does not possess it, according to its sex. This would require that the sex be determined already in the egg, independently from the spermatozoön and fertilization.

I consider this almost a logical consequence of the facts. It is possible, however, that, for example, the hologynic hemophilia is transmitted by something that is in all the eggs of an affected female, independent of sex, and that the male-determining spermatozoön destroys this thing. But this mechanism is entirely different from the chromosomal mechanism which explains the heredity of the diagynic hemophilia or of the not-sex-linked dominant hemophilia. It seems too improbable that the same character can be transmitted by two entirely different mechanisms; we can suppose that in one family one chromosome transmits it, in another family another chromosome, but the mechanism of the transmission must be the same in all cases. Therefore, there should be two kinds of eggs, and two kinds of spermatozoa, in order to explain the diagynic heredity.

This hypothesis requires a selective fertilization; the male-determining egg must be fertilized by a male-determining spermatozoön and the female-determining egg by a female-determining spermatozoön.

The following scheme will explain this type of heredity: Let F be the chromosome which always remains in the female line; so that a pair, Ff, in the female corresponds to a pair, ff, in the male; and let M be the Y chromosome and m the X chromosome. Then a mating will result in the following combinations, the impossible combinations being written in parenthesis:

P_1	Mmff	mmFf
Germ cells	Mf or mf	mF or mf
F_1	Mmff or (MmFf)	mmFf or (mmff)

The hologynic character is transmitted by F. This scheme allows also for the possibility of a holandric character transmitted by M.

The diagynic character is transmitted by m; the scheme provides also for a dia-andric transmission which can be made by f.

This scheme can also be stated in a simpler form as follows:

P_1	m—	mmF
Germ cells	m or —	mF or m
F_1	m or (mF—)	mmF— or (mm—)

This is the simplest symbolic expression of sex-linked heredity in man (diagynic and hologynic). But M is also necessary to explain the holandric heredity, and this is permitted by the type of sex chromosomes which was described in man by WIEMAN (1917).

If we consider sex-linked heredity in a general way, we are surprised by the circumstance that in some insects the female is heterozygous (e. g., in the Lepidoptera), while in all the others the male is heterozygous. It seems that the heterozygous constitution of the male is the more general condition in animals. Heterozygous females are observed only in the Lepidoptera and in birds. This difference, even in the same class, is very difficult to explain; we cannot understand why the chromosome pair XX of the female and the single X chromosome of the male can be replaced by an inverted condition. But if we take the hypothesis of an Mmff group in the male and an mmFf group in the female as a primitive arrangement, we can easily explain the reverse cases. It is possible that in some species certain chromosomes have disappeared before reduction, in which case the possibility of certain kinds of heredity has been lost.

Is the hypothesis of a selective fertilization a very improbable one? Some other facts seem to support the same hypothesis.

In the great fly, *Calliphora erythrocephala*, the sex ratio is exactly 1 : 1 in the best conditions of life. The experiments of Mrs. VALENTI (1913) with many isolated couples of this species have demonstrated that the sons and the daughters of one couple are too often exactly equal in number, or almost equal, than would result from statistical law, and there must be a mechanism by which this result is obtained. We can assume that the mechanism consists in a sexual division, namely, in the production of a male egg and a female egg from a neutral cell; then the eggs of both sexes are equal in number and if they all develop, the sons and the daughters are also equal in number. On the other hand, it is known that in flies the male is heterozygous, and that consequently there is an equal number of male and female spermatozoa; a selective fertilization is the logical corollary of these facts.

I must admit that from seeking an explanation of sex-linked heredity in man, I have been led to the hypothesis of a selective fertilization, a result which has exceeded my original purpose. Although it is a logical consequence, I express it, nevertheless, with some reserve, for I know how dangerous and doubtful is the application of logic to experimental science when data are still inadequate. Future researches may be expected to decide the question.

It is remarkable that a hologynic or diagynic character in man can also be transmitted in a more ordinary way as a dominant one, independent of sex. No sex-linked character is known in man, which behaves as a sex-linked character in all the families in which it appears. Hemophilia is the classical instance of diagynic heredity; now we also know two short pedigrees of hologynic hemophilia, and a pedigree of dominant hemophilia. Many affections of the eye follow the diagynic method of heredity; but some pedigrees are hologynic and others non-sex-linked dominant; e. g., nystagmus, myopia (?), daltonism, hemeralopia, neuritis optica. Cataract has been known only as a dominant character. Now a case of hologynic cataract is discovered.

The explanation of this variability is very simple if the chromosome scheme formulated above is accepted. The character can be transmitted by one chromosome or by another chromosome; there are three possibilities, the chromosome m, F or an autosome. So far as is known, the character is always transmitted, in any given family, by the same chromosome. So we can enunciate three principles which seem to apply to the sex-linked characters in man:

1. Sex-linked characters in man can also be transmitted as ordinary non-sex-linked dominants.
2. Sex-linked heredity in man is diagynic or hologynic; some characters (all?) are transmissible as hologynic and as diagynic. Perhaps a holandric heredity also exists.
3. In a single family, each sex-linked character is transmitted according to a constant method (by the same chromosome). This principle needs further confirmation.

Each sex-linked character of other animals, so far as is known, is transmitted only according to a single rule, always as sex-linked.

Hologynic heredity is known only in man.

The third principle, if confirmed, will constitute a further proof of the individuality of the chromosomes in man.

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AN ATTEMPT TO IMPROVE THROUGH SELECTION THE STYLE LENGTH AND FERTILITY OF *OENOTHERA BREVISTYLIS*[1]

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In an earlier paper (Davis 1918) there were published some observations on a selfed line of *Oenothera brevistylis* which had been carried for two generations through plants selected for greater variation in the direction of normal styles and stigmas. The line has now passed through five generations of selection, and not only has there been no improvement but the generations of later years have shown a retrogression so positive as to lend no encouragement to further effort. Apparently the variations in style length and fertility of *Oenothera brevistylis* are not due to heritable factors and consequently there can be no permanent success in selection for the improvement of these characters.

The history of the first two generations has been published in detail (Davis 1918, pp. 520–524) and requires but brief review. *Oenothera brevistylis* differs from *Oe. Lamarckiana* chiefly in its short style and reduced stigma which generally lies near the top of the calyx tube instead of at a point above the tips of the anthers. With this poor development of the style and stigma is correlated a very low degree of seed production, the ovaries ripening few or no seeds. There is, however, much variation among different plants of *brevistylis* and between flowers on the same plant with respect to the length of style, and occasionally the stigma will be present at the level of the lower tips of the anthers or somewhat above them. In these flowers the stigma lobes are generally found to be more normal in size and appearance. Although such styles are far from the full length which in *Lamarckiana* is above the anthers, they nevertheless do present a marked variation in that direction and suggest the possibility of selection for permanent improvement of this character. Such longer-styled flowers with more perfect stigmas have a distinctly greater degree

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[1] Genetical studies on Oenothera XI. Contribution from the Botanical Laboratory, University of Michigan, No. 188.

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of fertility and it is possible by persistent selfing, in spite of many failures, to obtain from them a few seeds.

The selection of an individual in each generation to carry on the selfed line was based on a test of performance of all of the plants in each culture. The test, introduced with the second generation, consisted in recording the length of style in the series of flowers opening along the main stem of each plant over a definite period of time. After the first generation the styles were listed as in three grades, *long* when the stigma reached above the lower tips of the anthers, *medium* when the stigma lay between the lower tips of the anthers and the top of the calyx tube, *short* when the stigma was at or below the top of the calyx tube. A full record of the performance of the plants in the first and second generations of the selfed line is given in tables 6 and 8 of the paper cited (DAVIS 1918) and will not be repeated.

The best individual, 16.2d, IV-4, from the first generation of 8 plants, showed a record between July 18 and August 3, 1916 of 19 long-styled and 0 short-styled flowers, a percentage of 100. In this first generation styles were recorded as long when they extended well above the top of the calyx tube and as short if the stigma was at or below the level of the calyx tube. There were selfed 14 flowers of this best individual which set 8 capsules with a total of 104 seeds. The poorest record of this generation was that of a plant which bore 9 long-styled and 7 short-styled flowers over the same period of time and on which 12 selfings gave 7 capsules with a total of 18 seeds.

The second generation consisted of 33 plants from the 104 seeds set by the selected individual, 16.2d, IV-4, of the first generation, the performance of which is given in the paragraph above. There was a wide range of performance in this generation between June 30 and July 28, 1917. The culture presented 6 plants with high percentages of long-styled flowers as follows, 76.9, 75.0, 73.5, 69.2, 65.5 and 64.7, but none of these was chosen as parent for the third generation because they gave either no seed when selfed or the yield was small (DAVIS 1918, table 8). The plant selected to carry forward the selfed line, 17.2, V-1 ripened 54 seeds from 11 capsules after selfing 24 flowers, and produced over the period of observation 19 long-styled, 9 medium-styled and 3 short-styled flowers, a record of 61.3 percent of long-styled flowers. The poorest plant of the second generation bore 0 long-styled, 12 medium-styled and 19 short-styled flowers. The record of the culture as a whole was much below that of the first generation, as indicated in table 7, although the two generations cannot be compared with strict accuracy because of the different system of recording style length introduced with the second generation.

The parent of the third generation was 17.2, V-1 described in the preceding paragraph. Its seeds, being soaked in water 24 hours, were forced to complete germination in a Petri dish following alternate exhaust and pressure up to 75 pounds applied 4 times in the course of 24 hours. As shown in table 1 the 54 seeds from 11 capsules gave 11 seedlings, a germination percentage of 20.4, and 10 *brevistylis* plants reached maturity.

The 10 plants of the third generation (table 2) made a record somewhat worse than that of the second generation and was far below that of the first. Its best performance, by plant 19.2, I-3, from July 7 to July 25, 1919, was 23 long-styled, 9 medium-styled and 4 short-styled flowers, a record of 63.9 percent long-styled flowers. There were selfed 21 flowers which set only 5 capsules with a total of 15 seeds, a very low degree of fertility. The second-best plant in the culture produced 20 long-styled, 8 medium-styled and 6 short-styled flowers over the same period of time, a percentage of 58.8 long-styled flowers. As shown in table 2 the remaining 8 plants of the culture had percentages of long-styled flowers ranging from 3.3 to 28.6, all very bad records.

TABLE 1

CULTURE	PARENT PLANT *brevistylis*	SEEDS SOWN	NUMBER OF SEEDLINGS	PERCENTAGE OF GERMINATION	PLANTS REACHING MATURITY
19.2	17.2, V-1	54 11 capsules	11	20.4	10

The best plant of the third generation, 19.2, 1–3, was selected as parent of the fourth. Its 15 seeds from 5 capsules after selfing 21 flowers were forced to complete germination in Petri dishes after being soaked in water and following alternate exhaust and pressure up to 45 pounds. Complete germination gave 4 seedlings, of which 2 died early, so that only 2 plants reached maturity; the germination was 26.7 percent (table 3).

The fourth generation, therefore consisted of 2 plants the performance of which between July 12 and August 2, 1920 is given in table 4. The best of these plants, 20.2–1, with 7 long-styled, 16 medium-styled and 15 short-styled flowers made a record of only 18.4 percent long-styled flowers. From 25 selfed flowers 7 capsules were set which ripened 18 seeds. The other plant presented a record of 13.9 percent long-styled flowers.

The 18 seeds from the best plant of the fourth generation, 20.2–1, after 24 hours in water, were subjected to alternate exhaust and pressure up to 30 pounds 10 times in 24 hours. Complete germination yielded 8 seedlings, a percentage of 36.4 (table 5); 6 plants reached maturity.

TABLE 2

Performance in the third generation of brevistylis in a selfed line. Observations from July 7 to 25, 1919.

PLANTS	NUMBER OF FLOWERS OBSERVED	LONG-STYLED	MEDIUM-STYLED	SHORT-STYLED	PERCENTAGE OF LONG-STYLED FLOWERS	YIELD OF SEED FROM SELFED FLOWERS
19.2, I–3	36	23	9	4	63.9	21 flowers selfed gave 5 capsules with 15 seeds
19.2, II–3	34	20	8	6	58.8	
19.2, I–5	35	10	11	14	28.6	
19.2, I–4	32	8	15	9	25.0	
19.2, II–4	30	7	9	14	23.3	
19.2, I–2	32	5	10	17	15.6	
19.2, II–5	35	5	14	16	14.3	
19.2, II–1	24	3	5	16	12.5	
19.2, I–1	33	3	10	20	9.1	
19.2, II–2	30	1	9	20	3.3	

TABLE 3

CULTURE	PARENT PLANT *brevistylis*	SEEDS SOWN	NUMBER OF SEEDLINGS	PERCENTAGE OF GERMINATION	PLANTS REACHING MATURITY
20.2	19.2, I-3	15 5 capsules	4	26.7	2

TABLE 4

Performance in the fourth generation of brevistylis in a selfed line. Observations from July 12 to August 2, 1920.

PLANTS	NUMBER OF FLOWERS OBSERVED	LONG-STYLED	MEDIUM-STYLED	SHORT-STYLED	PERCENTAGE OF LONG-STYLED FLOWERS	YIELD OF SEED FROM SELFED FLOWERS
20.2–1	38	7	16	15	18.4	25 flowers selfed gave 7 capsules with 18 seeds
20.2–2	36	5	11	20	13.9	

The performance of the 6 plants in the fifth generation (table 6), taken between July 20 and August 9, 1921, was somewhat better than that of the fourth, but not so good as that of the third, and it was far below the records of the first and second generations. Its best plant, with 14 long-styled, 13 medium-styled and 10 short-styled flowers, made a record of 37.6 percent long-styled flowers as contrasted with records of 76.9 percent and 63.9 percent for the best plants of the second and third generations, respectively. The other five plants of the culture had records for long-styled flowers ranging from 22.2 to 9.7 percent (table 6), which were very poor performances.

TABLE 5

CULTURE	PARENT *brevistylis* PLANT	SEEDS SOWN	NUMBER OF SEEDLINGS	PERCENTAGE OF GERMINATION	PLANTS REACHING MATURITY
21.2	20.2–1	18 (7 capsules)	8	36.4	6

TABLE 6

Performance in the fifth generation of brevistylis in a selfed line. Observations from July 20 to August 9, 1921.

PLANTS	NUMBER OF FLOWERS OBSERVED	LONG-STYLED	MEDIUM-STYLED	SHORT-STYLED	PERCENTAGE OF LONG-STYLED FLOWERS
21.2–6	37	14	13	10	37.6
21.2–3	36	8	16	12	22.2
21.2–2	33	6	13	14	18.2
21.2–5	35	6	15	14	17.1
21.2–1	39	6	18	15	15.4
21.2–4	31	3	12	16	9.7

Thus instead of improvement in the length of style as the result of selection for these five generations there took place a marked retrogression. The records of the third, fourth and fifth generations were far below those of the first and second. This retrogressive movement is clearly expressed in table 7 which gives the distribution of the plants for all generations grouped in percentages of long-styled flowers. There is no suggestion in this table of probable or permanent advance in style length.

As has been stated, there is correlated with the poor development of the style and stigma of *Oenothera brevistylis* a very low degree of seed production. On this subject it is difficult to obtain much data because so few capsules set any seed that a very large number of pollinations must be made to obtain harvests worth consideration. In choosing parent plants for the five generations of this selfed line it was of course necessary to pick individuals that produced enough seed to ensure a progeny and in some cases plants with better records of performance in style length could not be used because they ripened either no seed at all or so few seeds that there was no prospect of a satisfactory succeeding generation. This was particularly true of the large second generation (DAVIS 1918, table 8) in which for this reason 6 plants were discarded although their percentage records of long-styled flowers were somewhat higher than that of the plant selected to carry forward the line.

TABLE 7

Distribution of the plants of brevistylis through 5 generations, grouped as to the percentages of long-styled flowers.

PERCENTAGES IN CLASSES	GENERATIONS				
	First	Second	Third	Fourth	Fifth
0–10		3	2		1
11–20		5	3	2	3
21–30		6	3		1
31–40		7			1
41–50		3			
51–60	1	2	1		
61–70	1	4	1		
71–80	2	3			
81–90	2				
91–100	2				
	8	33	10	2	6

Table 8 gives the comparative fertility of the plants selected as parents of the five generations of this selfed line of *brevistylis*. It presents a record consistent with that of table 7, showing that fertility fell off in the later generations as did the percentages of long-styled flowers. Thus the average number of seeds per capsule in those set by the five parent plants were 6.6, 13.0, 4.9, 3.0 and 2.6. The percentages of selfed capsules that set seed likewise declined with the later generations, e.g., 35.7, 57.1, 45.8, 23.8 and 28.0. The percentages of germination fluctuated without apparent significance. These conclusions, because of the very low fertility, are necessarily based on a small amount of data, but it is a matter of interest that they show a close correlation between higher degrees of fertility and higher percentages of long-styled flowers.

TABLE 8

Comparative fertility of the plants selected as parents of the five generations of this selfed line of brevistylis.

SELECTED PLANT	NUMBER OF FLOWERS SELFED	NUMBER OF CAPSULES THAT SET SEED	TOTAL NUMBER OF SEEDS	PERCENTAGE OF SELFED CAPSULES THAT SET SEED	AVERAGE NUMBER OF SEEDS PER CAPSULE	PERCENTAGE OF GERMINATION
Parent plant of the selfed line 15.15d, I–1	14	5	33	35.7	6.6	48.5
From first generation 16.2d, IV–4	14	8	104	57.1	13	34.6
From second generation 17.2, V–1	24	11	54	45.8	4.9	20.4
From third generation 19.2, I–3	21	5	15	23.8	3	26.7
From fourth generation 20.2–1	25	7	18	28	2.6	36.4

Thus after a five-years study of a selfed line of *brevistylis* I am brought to the conclusion that the factors which are responsible for the reduced style length and stigma structure in this plant, and with the correlated very low fertility, are fixed, in the sense that they express themselves through fluctuations within distinctly limited ranges. The lower ranges are close to the usual conditions in the plant, which are those of complete or almost complete sterility. The higher ranges may be found by close observations on performance and tests for fertility. They express a small but definite advance in style length and stigma structure and in somewhat increased fertility, but there is no evidence that such improvement will be inherited. Selections for increased style length and increased fertility failed to hold the gains and the results were so discouraging to further attempts to improve style length and fertility that this selfed line will not be continued.

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THE INTENSITY OF LINKAGE BETWEEN THE FACTORS FOR SUGARY ENDOSPERM AND FOR TUNICATE EARS AND THE RELATIVE FREQUENCY OF THEIR CROSSING OVER IN MICROSPORE AND MEGASPORE DEVELOPMENT[1]

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INTRODUCTION

In a previous paper (EYSTER 1920) concerned with the linkage relations of the factors for sugary endosperm and the tunicate character in maize, data were presented which show a linkage between the factor pairs $S_u\ s_u$ and $T_u\ t_u$, with an average crossover value of 29.15 ± 0.89 percent. A striking feature of these data was that crossing over seems to have occurred about eight percent more frequently in microsporogenesis than in megasporogenesis.

A statistical study of BREGGER'S (1918) data on the linkage between the factor pair for waxy endosperm, $W_x\ w_x$, and the $C\ c$ aleurone factor pair shows a similar but much less marked difference in the amount of crossing over in the production of microspores and megaspores. GOWEN (1919) pointed out that ALTENBURG'S (1916) data on the linkage of factors in *Primula sinensis* show that crossing over also occurred more frequently in microspore development than in megaspore development.

In order to obtain more critical evidence on the relative amounts of crossing over in the formation of microspores and megaspores, individual

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[1] The original crosses and back-crosses were made at CORNELL UNIVERSITY. I am indebted to that institution and especially to Dr. R. A. EMERSON for garden space and facilities for making the pollinations. The remainder of the work was done as a project of the Department of Field Crops, UNIVERSITY OF MISSOURI.

F_1 plants heterozygous for sugary endosperm and tunicate ear were crossed reciprocally with sugary non-tunicate plants. In the present paper are given the results obtained in the progenies of the reciprocal back-crosses.

EMERSON and HUTCHISON (1921) presented extensive data on the relative frequency of crossing over in microspore and megaspore development in maize. Their data include observations on factor linkages in two different chromosomes. The factor pairs B b and L_g l_g are in the same chromosome and about 37 units apart. Crossing over between these factor pairs was found to have occurred, on the average, 1.43±0.72 percent more frequently in microspore development than in megaspore development. This difference is small and probably not significant. The factor pairs C c and S_h s_h are located in another chromosome at loci which are approximately 3 units apart. In this chromosome crossing over between C c and S_h s_h was found to have occurred somewhat more frequently in megaspore development than in microspore development. From these data it was concluded that crossing over in maize is not greatly different in megaspore and microspore development.

LINKAGE INTENSITY BETWEEN THE FACTOR PAIRS S_u s_u AND T_u t_u IN MAIZE

A summary of the data previously reported on the linkage relation between the factors for sugary endosperm and tunicate ear in maize follows:

Non-crossovers		*Crossovers*		*Total*	*Crossovers*	*Percent crossovers*
S_u T_u	s_u t_u	S_u t_u	s_u T_u			
430	406	175	169	1180	344	29.15±0.89

These back-cross data represent a coupling series as the constitution of the parents in the original crosses was $\frac{S_u\ T_u}{S_u\ t_u}$ and $\frac{s_u\ t_u}{s_u\ t_u}$, respectively.

In table 1 are given additional back-cross data on the frequency of crossing over between the factors S_u s_u and T_u t_u. These data represent a repulsion series. The constitution of the parents in the original crosses were $\frac{s_u\ T_u}{s_u\ t_u}$ and $\frac{S_u\ t_u}{S_u\ t_u}$, respectively. A summary of the data recorded in table 1 follows:

Non-crossovers		*Crossovers*		*Total*	*Crossovers*	*Percent crossovers*
S_u t_u	s_u T_u	S_u T_u	s_u t_u			
1343	1121	547	433	3444	980	28.46±0.52

These results are in close agreement with the data previously reported as may be seen by comparing the summaries given above. There is a difference of only 0.69 ± 1.03 percent.

TABLE 1

Frequency of crossing over between $S_u\ s_u$ and $T_u\ t_u$ observed in the back-crosses

$$\frac{s_u\ T_u}{S_u\ t_u}\times\frac{s_u\ t_u}{s_u\ t_u}\ \text{and}\ \frac{s_u\ t_u}{s_u\ t_u}\times\frac{s_u\ T_u}{S_u\ t_u}.$$

PEDIGREE NUMBERS	$S_u\ T_u$	$S_u\ t_u$	$s_u\ T_u$	$s_u\ t_u$	TOTAL	CROSS-OVERS	CROSSOVER PERCENTAGE
1544–1545	45	145	144	51	385	96	24.94
1546–1547	23	84	53	32	192	55	28.65
1548–1549	40	104	96	25	265	65	24.53
1550–1551	25	62	52	29	168	54	32.14
1552–1553	55	84	38	32	209	87	41.63
1554–1555	27	65	53	24	169	51	30.18
1556–1557	55	167	125	46	393	101	25.70
1558–1561	81	163	148	44	436	125	28.67
1562–1563	32	66	66	16	180	48	26.67
1564–1565	37	96	89	24	246	61	24.80
1566–1567	37	93	67	37	234	74	31.62
1568–1569	60	117	97	25	299	85	28.43
1570–1571	30	97	93	48	268	78	29.10
Total	547	1343	1121	433	3444	980	28.46 ± 0.52

Following is a summary of the data on the linkage between $S_u\ s_u$ and $T_u\ t_u$, including both the coupling and repulsion series:

Non-crossovers	*Crossovers*	*Total*	*Percent crossovers*
3300	1324	4624	28.63 ± 0.45

The average crossover value is thus seen to be 28.63 ± 0.45 percent.

THE RELATIVE FREQUENCY OF CROSSING OVER BETWEEN $S_u\ s_u$ AND $T_u\ t_u$ IN MEGASPORE AND MICROSPORE DEVELOPMENT

As already indicated F_1 plants of the constitution $S_u\ s_u\ T_u\ t_u$ from the cross $\frac{s_u\ T_u}{s_u\ t_u}\times\frac{S_u\ t_u}{S_u\ t_u}$ were back-crossed reciprocally with non-tunicate plants having sugary endosperm. The results are listed in table 2. When the F_1 plants were used as seed parents the percent of crossing over varied from 26.67 ± 2.28 to 32.14 ± 2.36. When the same F_1 plants were used

TABLE 2

Crossing over between $S_u\ s_u$ and $T_u\ t_u$ as shown by the back-crosses

$$\frac{S_u\ t_u}{s_u\ T_u}\times\frac{s_u\ t_u}{s_u\ t_u}\ \text{and}\ \frac{s_u\ t_u}{s_u\ t_u}\times\frac{S_u\ t_u}{s_u\ T_u}.$$

PEDIGREE NUMBERS	F₁ PLANTS AS SEED PARENT							F₁ PLANTS AS POLLEN PARENT								
	$S_u\ T_u$	$S_u\ t_u$	$s_u\ T_u$	$s_u\ t_u$	TOTAL	CROSS-OVERS	CROSSOVER PERCENTAGE	$S_u\ T_u$	$S_u\ t_u$	$s_u\ T_u$	$s_u\ s_u$	TOTAL	CROSS-OVERS	CROSSOVER PERCENTAGE	DIFFERENCE	$\frac{\text{DIFF.}}{\text{P.E.}_{\text{diff}}}$
660–13	27	65	53	24	169	51	30.18±2.35	100	312	269	97	778	197	25.32±1.10	−4.86±2.59	1.88
660–16	32	66	66	16	180	48	26.67±2.28	81	163	148	42	434	123	28.34±1.47	+1.67±2.71	0.62
661–2	25	62	52	29	168	54	32.14±2.36	55	84	38	32	209	87	41.63±2.12	+9.49±3.17	2.99
663–6	60	117	97	25	299	85	28.43±1.77	30	97	93	48	268	78	29.10±1.87	+0.67±2.57	0.26
663–9	37	93	67	37	234	74	31.62±2.01	37	96	89	24	246	61	24.39±1.95	−7.23±2.80	2.58
Total	181	403	335	131	1050	312	29.71±0.95	303	752	637	243	1935	546	28.22±0.98	−1.49±1.36	1.10

as pollen parents the percent of crossing over varied from 24.39 ± 1.96 to 41.63 ± 2.12. A summary of these data follows:

	F_1 as seed parent	*F_1 as pollen parent*
Non-crossovers	738	1389
Crossovers	312	546
Total	1050	1935
Percent crossing over	29.71 ± 0.95	28.22 ± 0.98
Difference = 1.49 ± 1.36;	$\frac{\text{Diff.}}{\text{P. E.}_{\text{Diff.}}} = 1.10$;	P = 45.81

In megaspore development the average percent of crossing over was 29.71 ± 0.95 while in microspore development it was 28.22 ± 0.98. From these results it is to be concluded that crossing over in these five F_1 plants occurred on the whole 1.49 ± 1.36 percent more frequently in megaspore development than in microspore development. A deviation as large as this would be expected to occur about once in every two trials due to the errors of random sampling.

SUMMARY

A total of 4624 back-cross plants, representing both coupling and repulsion series of the factor pairs S_u s_u and T_u t_u, have been observed. The average percent of crossing over between these factor pairs was found to be 28.63 ± 0.45.

The same F_1 plants were used both as seed and as pollen parents in back-crosses with double-recessive plants to determine the relative frequency of crossing over in megaspore and microspore development. It has been found that crossing over between S_u s_u and T_u t_u is variable in both megaspore and microspore development and occurs in each process with approximately the same frequency. This is in agreement with the conclusion reached by EMERSON and HUTCHISON (1921) in their study of the relative frequency of crossing over in megaspore and microspore development in maize.

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